

Control of the Pars Intermedia
of the Lizard,
Anolis carolinensis

Leonard Larsson



Lund 1981

Control of the Pars intermedia of the Lizard

Anolis carolinensis

av

Leonard Larsson

Fil mag, Mlm

Akademisk avhandling som för avläggande av filosofie
doktorsexamen vid matematisk-naturvetenskapliga fakul-
teten vid universitetet i Lund kommer att offentligen
försvaras å Zoologiska institutionen, Helgonavägen 3 A,
Lund, onsdagen den 27 maj 1981 kl 10.15



Digitized by the Internet Archive
in 2026

From the Department of Zoology, University of Lund,
Lund, Sweden

Control of the Pars intermedia of the Lizard
Anolis carolinensis

Leonard Larsson

LUND 1981

To my family and my parents

Inger, Veronica, Tobias

Maja and Alexander



A book may be amusing with numerous
errors,
or it may be very dull without a
single absurdity

Oliver Goldsmith

This thesis is based on the following papers:

- I. Rodríguez, E. M., Larsson, L., Meurling, P.: Control of the pars intermedia of the lizard, Anolis carolinensis. I. Ultrastructure of the intact neural lobe. Cell Tiss. Res. 186, 241-258 (1978)
- II. Larsson, L., Rodríguez, E. M., Meurling, P.: Control of the pars intermedia of the lizard, Anolis carolinensis. II. Ultrastructure of the intact intermediate lobe. Cell Tiss. Res. 198, 411-426 (1979)
- III. Larsson, L., Rodríguez, E. M., Meurling, P.: Control of the pars intermedia of the lizard, Anolis carolinensis. III. Changes in the ultrastructure of the disconnected neuro-intermediate lobe. Cell Tiss. Res. 199, 1-23 (1979)
- IV. Larsson, L., Meurling, P.: Control of the pars intermedia of the lizard, Anolis carolinensis. IV. Transection of the pituitary stalk. Effects on colour changes and histology. Cell Tiss. Res. 204, 201-216 (1979)
- V. Larsson, L.: Control of the pars intermedia of the lizard, Anolis carolinensis. V. Extravascular transfer and cellular uptake of horseradish peroxidase in the neuro-intermediate lobe. Cell Tiss. Res. 214, 1-22 (1981)
- VI. Larsson, L.: Control of the pars intermedia of the lizard, Anolis carolinensis. VI. MSH release from allografted neuro-intermediate lobes and fine structure of the implanted tissue. (Manuscript)
- VII. Larsson, L.: Control of the pars intermedia of the lizard, Anolis carolinensis. VII. MSH release from allografted neuro-intermediate lobes under various experimental conditions. (Manuscript)

These papers will be referred to by their Roman numerals

CONTENTS

	Page
Preface	1
Results and comments on included papers	
I and II	2
III and IV	3
V	5
VI and VII	7
Conclusions	11
Acknowledgements	13

PREFACE

Mechanisms of melanophore stimulating hormone (MSH) release regulation have been studied in a great number of vertebrate species with the use of a variety of methods. The lizard Anolis carolinensis possesses a series of attributes which makes it particularly suitable for experimentation on MSH release regulation. It has a rapid and very pronounced colour change, which is readily observed and can be used as a parameter on circulating MSH levels. Colour change can be evoked in a predictable way. Melanophores have no direct sympathetic innervation but are solely controlled by humoral factors of which MSH regulates the background adaptation. The source of MSH, the pars intermedia of the hypophysis, is extremely well developed and contrary to other vertebrates, the pars intermedia of the lizard lacks direct innervation. This seems to be compensated for by numerous vessels originating mostly from the neural lobe artery. The regulation of MSH release appears to depend on a neurohaemal transmission probably exerted from the neural lobe.

Furthermore, the animal is easy to keep and stands experimentation well. There has also, over the years, accumulated a considerable knowledge about its physiology.

The present thesis comprises a series of studies on regulation of MSH release by means of various methods, viz. electron microscopy, histological techniques including a tracer technique and histochemistry, lesioning and transplantations.

RESULTS AND COMMENTS ON INCLUDED PAPERS

Morphological Considerations on the Neuro-intermediate Lobe in Intact Animals (I, II)

Neural Lobe. The neural lobe in Anolis carolinensis belongs to the primitive "amniotic type" i.e. it is sac-like but folded into 4-6 diverticulae. It is closely attached to the large intermediate lobe, but separated from it by a continuous vascular septum. There are no nerves passing this septum into the intermediate lobe. The ependymal, fibrous and external layers are readily recognized. Ependymal cells extend radiating processes, forming end-feet at the perivascular septum (PVS). Numerous profiles of proposed aminergic type reach the basal lamina. Peptidergic profiles (probably three types) are found mostly in the fibrous layer, away from direct contact with the PVS. Their position raises questions about the route of release for their secretory products. Ependymal end-feet form a non-continuous layer along the PVS.

Intermediate Lobe. Secretory cells of the intermediate lobe are arranged in lobules surrounded by the PVS, the latter carrying blood vessels from the neural lobe. Sparse stellate cells are found in the lobe and send narrow extensions between secretory cells. These extensions interpose to some extent between the PVS and secretory cells. Most secretory cells are filled with dense secretory granules. After stimulation of MSH release (moving the animals to a dark background) some cells are depleted of their dense granules, leading to a marked

proliferation of the rough endoplasmic reticulum (RER) and an increased extension of the Golgi complex. There is a striking difference between neighbouring cells concerning their response to such stimulation. In very active cells, scattered dense inclusions appear within the RER. Large cisternae are found in secretory cells especially after increased MSH release. Some cisternae appear to communicate with the extracellular space.

We found, in contrast to an earlier work by Forbes (1972) (for ref. see paper II), a significantly greater number of dense secretory granules compared to pale granules of secretory cells. Fragmentation of cytomembranes, indicative of apocrine secretion was not seen here. Further, the number of the so-called dark cells was insignificant in our material under intact conditions. We believe that dark cells are indicative of a degenerative process.

Transection of the Pituitary Stalk, Effects on Colour Change and Histology (III, IV)

Transection of the hypophysial stalk is followed by three phases of different chromatic behaviour of the animals. Initially, dark skin is retained, irrespective of background (MSH release is excessive) while during the second phase animals are constantly pale (insignificant MSH release). During the third phase, normal colour change is restored.

Neural Lobe. Aminergic profiles disappear rapidly (within the first day) from all parts of the lobe. Elimination of distal stumps of peptidergic profiles is seen at the second day after deafferentation. It is most accentuated in those areas close to the lesion, while peptidergic profiles in peripheral areas of the lobe are left visually unaffected for at least 10 days. Ependyma exhibits macrophage activity during elimination of neurons, by extending pseudopodia-like processes and engulfing pieces of axons. Degradation seems to occur particularly in phagosomes of the ependymal cytoplasm, but initially there appears to be also autolytic degradation of axons not engulfed by glial cells. The findings at the ultrastructural level correlate well with the distribution of aminergic (Falck-Hillarp technique) and aldehyde fuchsin (AF) positive fibres in the light microscope.

Restored vascular contact appears in most cases within the first week and during the third phase (restored normal colour change) there is a reinnervation of at least AF-positive fibres across the transected area into the remaining parts of the neural lobe. Intimate contacts are sometimes seen between capillaries and AF positive fibres outside the former neural lobe.

Intermediate Lobe. The great majority of secretory cells of this lobe does not appear adversely affected by lesioning the hypophysial stalk, but seemed rather markedly activated. There are outstanding structural evidences that the formation of secretory material is stimulated by the lesion, probably as a direct consequence of the excessive MSH release. An increased number of large cisternae in the secretory cells seems to correlate with the rapid MSH release. Hypothetically,

these cisternae might represent a morphological result of the rapid secretion and constitute one step in the recycling of cytomembranes.

During the period of slow release (second phase) the formation of new secretory granules proceeds until secretory cells are refilled. Synthesis of secretory material thus seems to be regulated by an intracellular mechanism, which might be triggered by the degree of stored secretory material.

Considering the great stores of MSH during the pale phase, one would question the nature of the release inhibition (or abolished stimulation) that occurs during this phase.

Morphological Basis for Extravascular Communication within the Neuro-intermediate Lobe (V)

Previous studies have accentuated the necessity to acquire extended knowledge of the morphological substrate for communication within the neuro-intermediate lobe (NIL). What is the significance of the localization of peptidergic profiles mainly away from vascular contact in the neural lobe (NL)? To what extent are ependymal end-feet transport barriers towards the perivascular space (PVS)? Is there a communication between the "cisternae" of the intermediate lobe (IL) and the inter-cellular space (ICS)? What is the possible route for newly released MSH into the main circulation? What is the fate of membranes of secretory granules?

The intravascular injection of the protein tracer horseradish peroxidase (HRP) in vivo is followed by a rapid distribution of the tracer to the PVS and the ICS of the two lobes. The penetration of HRP is, however, arrested at the level of intercellular junctions in the ependymal layer, suggesting a penetration barrier towards the cerebrospinal fluid.

Neural Lobe. The presence of micropinocytotic vesicles (MPV) and small endocytotic vacuoles (EV) in the apical ependymal cytoplasm 13 min after the start of the very slow injection suggests a rapid penetration of HRP to the ICS. The number of HRP containing cellular inclusions was increased during the next 24 hours. MPV attached to the cytomembrane, or MPV lying freely in the cytoplasm are seen contemporaneously with EV of different sizes. Dense bodies, multivesicular bodies and large (hetero?)phagosomes are found predominantly in the apical cytoplasm.

Labelled vesicles, comparable to MPV, are to be found in nervous profiles of the external layer.

After transection of the hypophysial stalk, numerous dense, labelled droplets are seen in peptidergic terminals. There also appears an increased number of labelled inclusions in glial cells.

Intermediate Lobe. Cisternae of the intermediate lobe secretory cells rapidly fill with the tracer, and are thus suggested to be in open communication with the ICS. Multilamellar inclusions are seen occasionally in these cisternae. Cisternae appear to be a consequence of rapid release of large stores of secretory granules and one step in recycling of membranes. It could not be excluded that they are pinched off from ICS contact in later stages, forming phagosomes.

Sparse MPV and EV are seen in secretory and stellate cells. Their number seems to increase during the next 24 h. Secretory cells appear to contain more numerous labelled inclusions of EV-type after stalk transection. Surprisingly, there is no significant aggregation of these EV into larger lysosomal complexes during the 24 h period.

The number and character of labelled cellular inclusions in stellate cells are seemingly unaffected by the stalk transection.

Survival and MSH release from Allografted Neuro-intermediate Lobes (VI, VII)

MSH release. A majority of grafted neuro-intermediate lobes release significant amounts of MSH during the initial period of implantation. The presence of MSH is disclosed by the hormone's action on melanophores above the implant in hypophysectomized recipients. Excessive spontaneous release of MSH ceases in most cases after some hours or a day. An insignificant dark skin patch thereafter remains above the implant. The graft responds to certain kinds of external stimulation by a marked increase of its MSH release. This is manifested by growth of the skin patch and frequently an overall darkening of the skin. The MSH reaches the main blood by subcutaneous vessels, which come into contact with the implant. These blood vessels could be identified from outside as dark striae radiating from the implant, because of their great content of MSH.

The change in darkness and skin patch diameter is suggested as a very sensitive parameter of MSH release, suitable for the evaluation of possible direct effects on MSH release of various substances. The grafts responded to external stimulation for extended time (more than 4 months).

Morphological Aspects on Survival of the graft. There appears to be a loss of tissue volume initially that hits the neuropil of the neural lobe as well as secretory cells of the intermediate lobe. It is reasonable that impaired nutrition at the ectopic site is a major cause. After some days the loss of tissue is impeded probably as a result of a restored blood supply. The cytology of remaining secretory cells, stellate cells and ependyma is virtually identical to the intact condition for extended periods (at least for 2 months for MSH cells). Peptidergic profiles could occasionally be identified in the former neural lobe for 3 weeks.

Immunological rejection of the implant proceeded in a slow rate in most implants. In late stages the formation of connective fibrils may be impressive. Host leucocytes including macrophages cluster around the implant and appear to ingest fragments of the implanted tissue. Secretory cells, although surrounded by macrophage pseudopodia seemingly retain their cytological integrity. In advanced stages they become depleted of secretory material.

Use of the Allografted Neuro-intermediate Lobe in the Search for Factors Affecting the MSH release. Excessive spontaneous release is confined to a period of some hours or a day after the implantation.

As has been reported above, spontaneous release of MSH has a time-limited duration also after transection of the hypophysial stalk (III, IV). There are conclusive evidences that diminished release activity is not caused by deficiency of secretory material. It appears to be important to reconsider the hypothesis that MSH release in Anolis is regulated entirely by an inhibitory mechanism.

It is shown here (in accordance with other authors) that serotonin exerts a very marked stimulation on MSH release by a direct action on the secretory cells. Furthermore, it is stated that distressing handling and adrenaline stimulate MSH release to a lesser degree. Although this latter effect has been known for some years, it is sometimes overlooked in the literature. Adrenaline stimulates MSH release via beta-adrenergic receptors and the effect could be blocked reversibly with propranolol.

Although the number of experiments with oxytocin and its derivatives in the present study was too small to allow extensive conclusions, there was no support for the opinion that these factors have any important effect on MSH release.

A trial was made to inhibit the MSH release during the initial period of excessive release using dopamine, which has been designated an MSH release inhibiting capacity. There was no marked decrease of MSH release after the administration of this amine, however. At least four possible explanations emerge: dopamine has no inhibitory effect on MSH release under the referred conditions; passive flow of MSH due

to tissue damage; dopamine affected the skin directly (3 cases out of 4); rapid metabolism of dopamine (despite stabilization). The first alternative is supported by Levitin (1980) (for ref. see paper VII).

The use of allografted neuro-intermediate lobes as described here appears to be a valuable complement to other methods in the study of regulation of MSH release.

CONCLUSIONS

1. The cytology of secretory cells of the intermediate lobe alternate between two phases of activity - a storage phase with numerous secretory granules and a synthesis phase with extensive rough endoplasmic reticulum.
2. Synthesis of MSH is probably regulated by an intracellular mechanism, triggered by the depletion of secretory materials.
3. The protein horseradish peroxidase penetrates rapidly the interstices of the neuro-intermediate lobe, suggesting possible transport routes for endogenous materials.
4. Allografted neuro-intermediate lobes exhibit sustained vitality despite activation of the recipient's immunological defence.
5. Grafted neuro-intermediate lobes could be used to test qualitatively and semiquantitatively the effects of various factors on MSH release.
6. There is a spontaneous release of MSH during a short period after transection of the hypophyseal stalk or after moving the neuro-intermediate lobe to ectopic sites — MSH release is thereafter very sparse, despite large stores of secretory granules.
7. Grafted neuro-intermediate lobes respond to serotonin (5-HT) and adrenaline (or distressing handling) by increasing MSH release.
8. Adrenaline stimulate MSH release to a lesser extent than 5-HT.
9. MSH release regulation is probably a complex matter which seemingly involve stimulatory as well as inhibitory mechanisms.

10. Regulation of MSH release in Anolis seems to differ from other vertebrates which have been studied, because the inhibitory element in the regulation appears of lesser importance.
11. It remains to be solved whether MSH release is triggered predominantly by 5-HT stimulation under intact conditions.

ACKNOWLEDGEMENTS

I greatly acknowledge the former director of the Division of Structural Zoology, prof. Erik Dahl and the present director prof. Rolf Elofsson for stimulation throughout the years and for laboratory facilities and Dr Patrick Meurling for initiating the investigation and for his indispensable support and criticism. His neverfailing inventiveness in social events and the great friendliness, consideratness and cooperation of him and all colleagues at the Department of Zoology has formed a fundament for mental health during the past decade. I am also greatly indebted to Dr Esteban Rodríguez (Valdivia, Chile) for very instructive cooperation and essential discussions on scientific and methodological topics.

Apart from my dear wife Inger and my children Veronica and Tobias, a heavy burden has been carried by the staff at the Division of Structural Zoology. I would like to express my gratitude to Mrs Lena Sandell, Mrs Ingrid Hallberg, Mrs Kirsten Thörneby, Miss Maria Walles and Mrs Rita Wallén for skilful technical assistance, to Miss Inger Norling and the late Mr Lajos Erdös for excellent photographic aid and to the engineer Folke Larsson for rediness to help and advise in technical subjects. For excellent typing and correcting manuscripts, Miss Ylwa Andersson is greatly acknowledged, as are Dr Curt Carlbom, Mr James Brown and Dr Edvin Brugge for careful linguistic aid and Mrs Gunilla Bergh and Miss Birgit Kjellberg for valuable office work. I am also very grateful to Mr Axel Hagström for excellent cooperation throughout the years and to Mrs Majken Elofsson for incomprehensible patience with the bad order in my room.

The director Malte Augrell and my colleagues at AB H Lundbeck & Co are greatly acknowledged for their significant support and kind consideration.

I would also like to express my gratitude to doc Anders Björklund and prof. Bengt Falck at the Department of Histology for stimulating and valuable criticism and for furnishing technical assistance in the Falck-Hillarp technique.

Finally, I am greatly indebted to the fascinating creature, Anolis without whose selfdevoting cooperation this thesis would have been impossible.

The investigations were supported by grants from the Swedish Natural Science Research Council (to Dr P Meurling) and grants from the Royal Physiographic Society of Lund.

Control of the Pars intermedia of the Lizard,
Anolis carolinensis

VI. MSH release from Allografted Neuro-intermediate
Lobes and Fine Structure of the Implanted Tissue

Leonard Larsson^{*}

Department of Zoology, University of Lund, Lund,
Sweden

Acknowledgements: The author is indebted to Mrs Lena Sandell and Miss Inger Norling for skilful technical assistance and photographic aid and to Miss Ylwa Andersson for excellent typing of the manuscript.

Doc Börje Karlsson is greatly acknowledged for valuable criticism.

^{*}Supported by grants from the Swedish Natural Science Research Council (to Dr P Meurling) and the Royal Physiographic Society of Lund

Summary. Melanophore stimulating hormone (MSH) release and morphology of allografted neuro-intermediate lobes, implanted subcutaneously into hypophysectomized lizards, (Anolis carolinensis) were studied maximally 124 days. Host rejection reactions, including proliferation of various leucocytes and activation of fibroblasts, were patent in most cases during later stages. Nevertheless, MSH release from the implanted tissue could be stimulated by various external stimuli for up to 124 days. Presence of MSH was visualized by the hormone's action on melanophores of the skin. Nonstimulated implants gave rise to a minute dark patch, indicating that basal release of MSH from the implant was slight. An enhancement of MSH release initiated growth and increased darkness of the skin patch, which overlaid the implant. Frequently, further MSH release caused a general skin darkening. Although the implants were continually exposed to the host's rejection reactions, they retained their gross morphological and cytological integrity over extended periods.

Key words: Neuro-intermediate lobe - Transplantation - Anolis carolinensis - MSH release - Ultrastructure

INTRODUCTION

The present paper deals with morphological aspects on survival of neuro-intermediate lobes allografted under the dorsal skin of the lizard, Anolis carolinensis. These grafts form the basis for a series of experiments concerned with the search for trophic factors in the regulation of melanophore stimulating hormone (MSH) release (Larsson, manuscript VII). One could expect that immunological reactions, as well as poor nutrition of the implant, would severely interfere with the implant's survival. It was, however, shown in a pilot study (Meurling and Larsson 1974) that neuro-intermediate lobes, under the referred conditions, can survive for long periods (more than 3 months) and evoke a darkening of the skin overlying the graft. Those findings inspired the use of such preparations to screen different substances for their possible direct effect on MSH release (Larsson, manuscript VII). Assaying the release of MSH from the intermediate lobe has thus far been mainly achieved under in vitro conditions by incubating the lobe and measuring the amount of MSH in samples of the medium by different bioassays and RIA methods. Provided that the MSH concentration is great enough, bioassays seem to give reliable quantitative information (Hadley et al. 1975, Thornton and Geschwind 1975a, b). But the in vitro technique mentioned above seems to involve some limitations as regards its suitability. It is laborious and requires a rather large number of experimental animals (otherwise, the concentration of MSH in the incubation medium is too low to be identified with any certainty). Further, this technique yields only limited information on the dynamics of MSH release after stimulation. But one should also consider the possibility that the

test substance may interfere with the bioassay. Such interference has to be disclosed in separate tests.

There seem to be no major disadvantages in incubating pituitary glands for long periods. Semoff et al. (1978) demonstrated a measurable MSH release from incubated intermediate lobes for up to 4 months in the frog, Rana pipiens. Upon ultrastructural examination of the incubates, they observed a well-preserved secretory cell morphology.

Transplantations have been frequently used in the study of hormone release from pituitary glands freed from direct hypothalamic influence. The effects of such dislocation on the release of most adenohipophysial hormones remains elusive for rapid evaluation (cf. van Dongen et al. 1966, Masur 1969).

The intermediate lobe/MSH system offers unique possibilities for direct estimation of hormone release, simply by observing the melanophore response of the skin. Levitin (1980a, b) showed an increased MSH release from hypophysial implants under the oral mucosa after systemic injection of serotonin. It has been shown that the chemical environment of the implant may affect the release of MSH under in vivo conditions. Iturriza (1969) demonstrated that MSH release from autotransplanted intermediate lobes in Bufo, grown in the anterior eye-chamber, was inhibited by the sympathetic innervation of the eye. Naturally, the most suitable condition for such experimentation is the use of autotransplants. Unfortunately, the relation of the internal carotids, deeply penetrating the intermediate lobe of Anolis,

raises technical obstacles for the extirpation of undamaged intermediate lobes in vivo. In the following, the conditions for the alternative use of allografts are evaluated.

MATERIALS AND METHODS

Adult male Anolis carolinensis VOIGT (Fam Iguanidae) were kept in 60-litre terraria containing brown gravel, pieces of bark, twigs and green plants. The ambient temperature ranged from 15° to 30° C. The lizards were fed Tenebrio larvae and houseflies and allowed to drink ad libitum. During the daytime (8 a.m. - 8 p.m.), the terraria were illuminated by carbon-filament lamps. The lizards were individually tagged with combinations of threads of different colours and lengths, stitched into the skin of the neck.

Hypophysectomy and Transplantation. Lizards used in the experiment were hypophysectomized prior to transplantation. The neuro-intermediate lobe was excised at the level of the infundibulum, leaving the median eminence attached to the hypothalamus. The distal lobe was replaced if possible in the hypophysial cavity. For details on approaching the pituitary gland, see Larsson et al. (1979b). No substitution therapy was given.

After hypophysectomy, the lizard's ability to adapt its skin colour in response to dark and light backgrounds was recorded during a subsequent period of minimally 3 days. Those exceptional animals,

exhibiting brownish skin postoperatively, were excluded from further experimentation. Pituitary glands were excised for implantation from freshly decapitated specimens and the distal lobe was carefully dissected from the neuro-intermediate lobe. Efforts were made not to damage the surrounding hypophysial capsule. The two lobes (neuro-intermediate lobe/distal lobe) were then grafted separately under the dorsal skin through a small incision. The implanted distal lobe served as a control. Recipients were lightly anaesthetized with ether. The neuro-intermediate lobe was placed behind the front leg (Fig. 1) and the distal lobe in front of the hind leg. The small lesion in the skin was covered with a piece of surgical sponge (Spongostan[®], Ferrosan).

Evaluations of the MSH Release and Subsequent Skin Darkening after Implantation. During initial stages of excessive MSH release, the overall skin darkening was assessed according to a five-point scale: G (green), BG (brownish-green), GB (greenish-brown), B (brown) and SB (supranormal brown, exhibited under experimental conditions only). For details of procedure, see Larsson and Meurling (1979). After an initial period of general skin darkening, a dark skin patch remained over the implant. The size of the patch, expressed as the average diameter, and its colour (judged according to the same above-mentioned scale) were recorded daily under a minimum of distress for the animals. The darkness and number of dark striae radiating from the implant were also recorded.

Effects of Pharmacological Treatment and Stressful Stimulation on MSH Release. The effects of various drugs, as well as of stressful stimulation on MSH release, were estimated by studying the size and darkness of the skin patch after subcutaneous injections of the test drug close to the implant. For details of procedure and results, see Larsson. (manuscript VII).

Electron Microscopy. The implanted neuro-intermediate lobes were excised at varying intervals and used for electron microscopy. They were fixed in iced glutaraldehyde and formaldehyde according to Karnovsky (1965), buffered to pH 7.5 with cacodylate buffer, and postfixed in 2% osmium tetroxide. Afterwards, they were dehydrated in a graded alcohol series, stained en bloc with phosphotungstic acid and uranyl acetate in absolute methanol and finally embedded in Vestopal. For details of procedure, see Larsson (1981). Ultrathin sections were examined in a Zeiss EM 10 electron microscope.

RESULTS

Functional considerations

Skin Effects. Implanted neuro-intermediate lobes initially released enough MSH to cause a general darkening of the skin. This reaction persisted from some hours up to about 1 day. In implants with a preserved hypophysial capsule, the period of general skin darkening appeared to be shorter. This may indicate an initial passive leakage of MSH from damaged secretory cells. Conceivably, the distal lobe (Fig. 1) contributes to this initial skin darkening by leaking ACTH (and MSH?). In later stages, the distal lobe implant produced an ill-defined dark skin patch, indicative of an ambiguous release of melanotropic substances. Exceptionally, a larger patch remained above the distal lobe, which most certainly was due to incomplete separation from the intermediate lobe.

The anterior skin patch, overlying the neuro-intermediate lobe usually exhibited an initial supranormal brown colour. The diameter averaged several mm (Fig. 1). Narrow, dark striae can also radiate from the skin patch, mostly in the direction of the axillar region. When present, they corresponded to blood vessels passing close to the implant and carrying MSH into the main blood circulation.

Normally, the anterior skin patch continuously diminished in size and appeared less dark during the first few days after the implantation; provided that the animal was not exposed to distressing stimulation, i.e. handling. In most cases, the size and darkness of the skin patch

RESULTS

Functional considerations

Skin Effects. Implanted neuro-intermediate lobes initially released enough MSH to cause a general darkening of the skin. This reaction persisted from some hours up to about 1 day. In implants with a preserved hypophysial capsule, the period of general skin darkening appeared to be shorter. This may indicate an initial passive leakage of MSH from damaged secretory cells. Conceivably, the distal lobe (Fig. 1) contributes to this initial skin darkening by leaking ACTH (and MSH?). In later stages, the distal lobe implant produced an ill-defined dark skin patch, indicative of an ambiguous release of melanotropic substances. Exceptionally, a larger patch remained above the distal lobe, which most certainly was due to incomplete separation from the intermediate lobe.

The anterior skin patch, overlying the neuro-intermediate lobe usually exhibited an initial supranormal brown colour. The diameter averaged several mm (Fig. 1). Narrow, dark striae can also radiate from the skin patch, mostly in the direction of the axillar region. When present, they corresponded to blood vessels passing close to the implant and carrying MSH into the main blood circulation.

Normally, the anterior skin patch continuously diminished in size and appeared less dark during the first few days after the implantation; provided that the animal was not exposed to distressing stimulation, i.e. handling. In most cases, the size and darkness of the skin patch

remained constant after a few days. The skin patch continued to be insignificant for long periods. After certain external stimulation (for details, see Larsson, manuscript VII) the skin patch was affected in the following manner (cf. Fig. 2): An intensively dark spot arose above the implant; the rapidity of formation depended on the type of stimulation. The patch increased in diameter and was sharply outlined against the surrounding pale green skin (2b-e). Predominantly distinct striae developed soon afterwards (Fig. 2c-e) and radiated from the patch mostly towards the axillar region. In some cases there were similar striae radiating from the posterior implant, which contained pars distalis cells. These striae orientated towards the inguinal region.

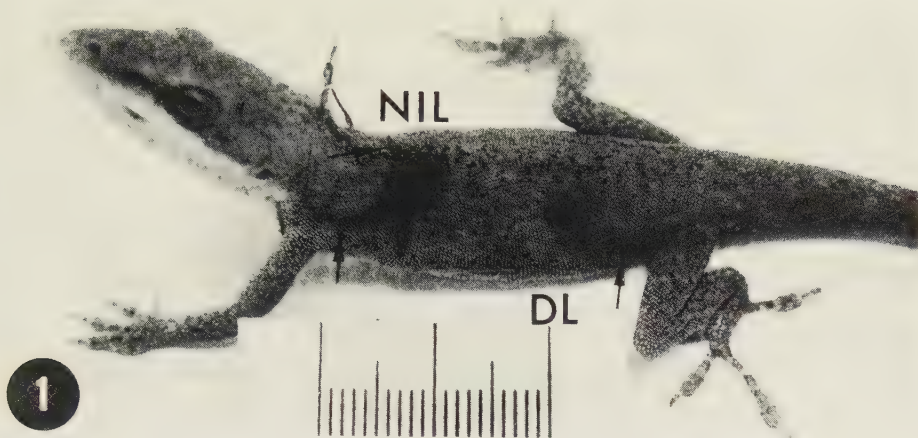
The sharp outline, and apparent difference in skin colour between the skin patch (supranormal brown) and the general paleness of the skin, persisted during initial phases of increased MSH release. Thereafter, the rapid growth of the skin patch was arrested and the border towards surrounding skin became less distinct (Fig. 2g). In this way, a skin patch developed with a darker centre and a marginal "diffusion" zone, exhibiting light brown colour. A similar situation appeared along the blood vessels (Fig. 2f). The gradual return to the initial condition was initiated by the disappearance of the dark "centre" of the radiating striae (Fig. 2h), and afterwards the dark central spot of the skin patch (Fig. 2k). The size of the skin patch persisted for varying periods.

The growth and regression of the skin patch following external stimulation was dependent on the establishment of new vascular

FIGURES 1 - 2
=====

Fig. 1. Skin-darkening in a hypophysectomized specimen 25 min after implantation of a neuro-intermediate lobe (NIL) and a distal lobe (DL). Note dark striae (arrows) directed towards the axilla and the inguinal region, respectively. They correspond to subcutaneous blood vessels. The lizards were individually tagged with threads, stitched into their necks. Bar indicates 20 mm

Fig. 2. Schematic representation of growth and remission of the skin patch, overlying the implanted neuro-intermediate lobe, after external stimulation. Dotted area, area exhibiting a skin colour paler than brown but darker than green; Solid areas, likewise indicate brown or supranormal brown colour. Time schedule varies among individuals. For details, see the text



relationships by the implant. After revascularization, the skin patch did not grow to great sizes, but instead there was a rapid distribution of MSH into the main blood circulation, causing a general darkening of the entire skin.

Survival of the Implant. The capacity of the implant to release MSH after external stimulation ceased at various intervals in the experimental animals. Most implants responded to external stimulation for 2 weeks or more. It appeared that impaired health of a hypophysectomized recipient was a more limiting factor for extended experimentation than was the survival of the implant. In two cases the vitality of the implants was clearly demonstrable after 77 and 124 days. In another implant with manifest vascular relations to the host, there was a marked release of MSH from the implant on the 53rd day of implantation. The amount of MSH in circulation was great enough to cause an overall brown skin. On the other hand a few implants rapidly became inactive and the implanted tissue was rejected. It appeared that this was due to improper handling of the tissue during implantation. There were no indications that the frequency of external stimulation (every second day) reduced the vitality of the implant. Table I illustrates the interval of functional vigour of individual implants.

Morphological Considerations

There was, among implanted neuro-intermediate lobes, a considerable variation with respect to cessation of MSH release, degree of

Table 1. Neuro-intermediate lobe implants

Total duration of experiment (weeks)	0-1	2	3	5	8	11	18	
Number of implants	7*	4	1	1	1	1	1	N = 16
Marked vitality at day of fixation	6	3	1	1	1	1	1	

* One recipient received a second implant on the contralateral side

revascularization, loss of implanted tissue, macrophage activity, and formation of collagen fibrils.

General Morphology. Most implants retained their gross morphological integrity for long periods (Figs. 3, 12). In the neural lobe there was a continuous elimination of the distal stumps of transected neurones, but peptidergic terminals could in one case still be identified 20 days after the implantation (Figs. 5, 7). Ependymal cells of the de-afferented neural lobe were orientated along the former ventricular lumen for extended periods (Figs. 3, 4) in a manner resembling the intact condition (cf. Rodríguez et al. 1978). In later stages the remains of the neural lobe consisted of one layer of ependymal cells standing directly on a basal lamina. Blood vessels, seemingly restored, were found in the interlobular connective tissue within the intermediate lobe, between separate lobules of the neural lobe, and in the bordering septum between the two lobes.

An abundance of collagen fibres (Figs. 4, 8) were seen in the perivascular septum and in the hypophysial capsule, together with various leucocytes, a large part of which seemed to be macrophages (Figs. 7, 9) (cf. Gibson 1979). In later stages the implants were surrounded by massive bundles of collagen fibres intermingled with various leucocytes. The macrophages frequently extended narrow pseudopodia into the hypophysial parenchyma. They penetrated primarily into the interlobular perivascular septum (PVS), but later on also interposed between adjoining cells (Fig. 8). Accordingly, lobules of the intermediate lobe were sequestered into smaller units. Single cells might lose contact with adjoining cells, round up and lie isolated surrounded by macrophage pseudopodia (Figs. 8, 11).

An initial loss of tissue appeared to occur in most implants that affected the neuropil of the neural lobe, as well as secretory cells of the intermediate lobe. The rate of elimination of secretory cells was later arrested in most cases, as judged from their production of secretion. This probably accorded with improved tissue nutrition by a process of revascularization. During more extended periods, there was a gradual, but rather slow, degradation of the implant, effectuated to a major extent by various macrophages. Necrotic areas were only rarely seen in implants surviving for periods longer than 2 weeks.

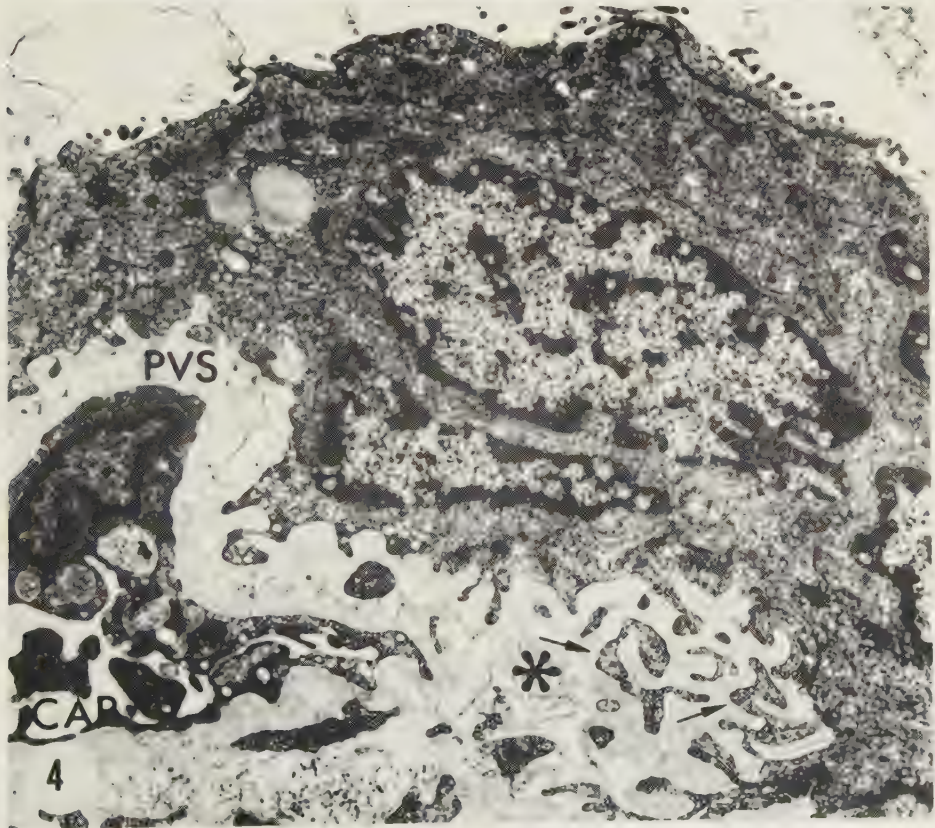
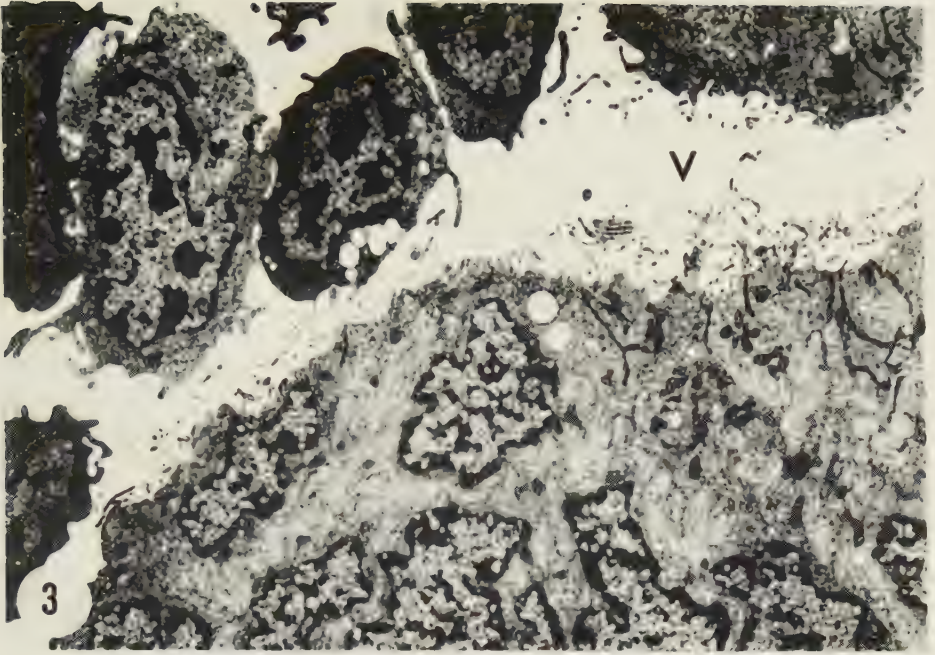
Intermediate Lobe. Secretory cells of the intermediate lobe retained their integrity for long periods. The number of secretory cells decreased, however, with varying rapidity among the implants. Thus, in one animal after 58 days of implantation (Figs. 10, 11), the cytology of the remaining cells closely resembled the intact condition

FIGURES 3 - 4

=====

Fig. 3. Neural lobe 37 days after implantation. The gross morphology is well preserved. Ependymal nuclei exhibit very dense chromatin. Nuclear pores are easily identified. The ependymal layer resembles the intact condition. Ependyma exhibits microvilli and scattered cilia into the former ventricular lumen (V), invaded by various leucocytes.
x 7 000

Fig. 4. Neural lobe, same implant as shown in figure 3. The neuropil is eliminated, leaving a single layer of ependymal cells. The perivascular septum (PVS) is thicker than usual and richly supplied with collagen fibres (asterisk). Fibroblast extensions penetrate into the PVS (arrows). CAP, capillary, seemingly occluded. x 14 400



(cf. Larsson et al. 1979a). Most cells were loaded with secretory granules, exhibiting a "storage phase". The cytoplasm contained sparse endoplasmic reticulum as compared with the "synthesis phase" (developed during excessive release of MSH). The Golgi complex was well developed in most cells. Secretory cells exhibited a varying number of clear vacuoles (Figs. 10-12) some of them resembling the cisterns discussed by Larsson et al. (1979a) and Larsson (1981). The vacuoles were either quite clear or contained a fine precipitate, which could be confined to the marginal zone of the vacuole.

The density and size of the secretory granules varied within the same limits as was found during in situ conditions (Larsson et al. 1979a). The nuclei, however, appeared more irregular than normal and exhibited deep enfoldings. The chromatin was markedly denser, especially along the nuclear membrane. Nuclear pores were easily discriminated. Nucleoli were large and were seen frequently.

In some areas the secretory cells were degranulated (Fig. 6), leaving a few dense granules and an abundance of pale, indefinitely limited granules. Cellular organelles were insignificant and the nuclei shrivelled. This condition obviously represented a stage of degeneration prior to complete elimination. Degranulated cells retained their cytomembrane for long periods. At least some of them were finally pierced by macrophages and ingested. Occasionally, stellate cells might appear as macrophages (Fig. 12).

External stimulation of MSH release (see Larsson, manuscript VII) - brought about by, i.e. handling or by administration of stimulant

FIGURES 5 - 7
=====

Fig. 5. Neural lobe 20 days after implantation.

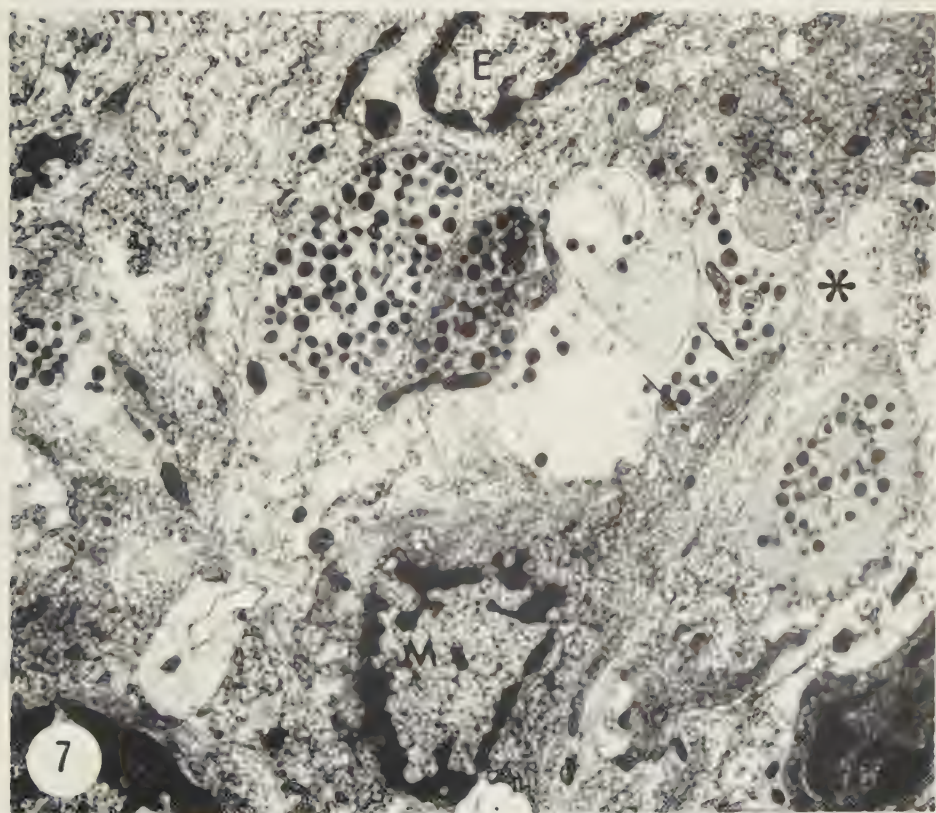
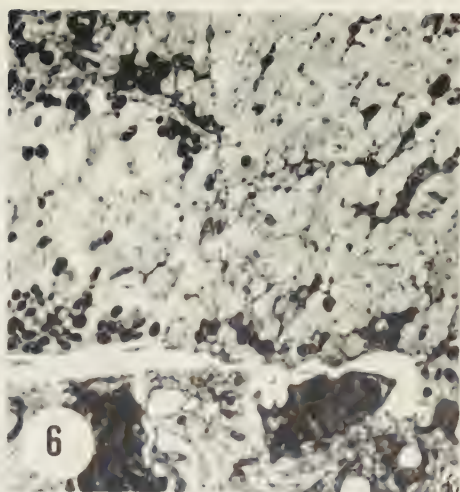
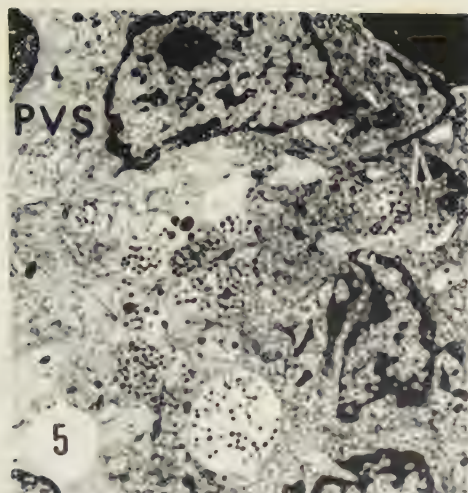
Scattered peptidergic-type nervous profiles persist in the former fibrous layer. Arrow, a pseudopodium from a host macrophage penetrating between the ependymal cells. PVS, perivascular septum. x 4 700

Fig. 6. Intermediate lobe 20 days after implantation.

Secretory cells are markedly degranulated. The implant is surrounded by host leucocytes.
x 5 800

Fig. 7. Neural lobe 20 days after implantation.

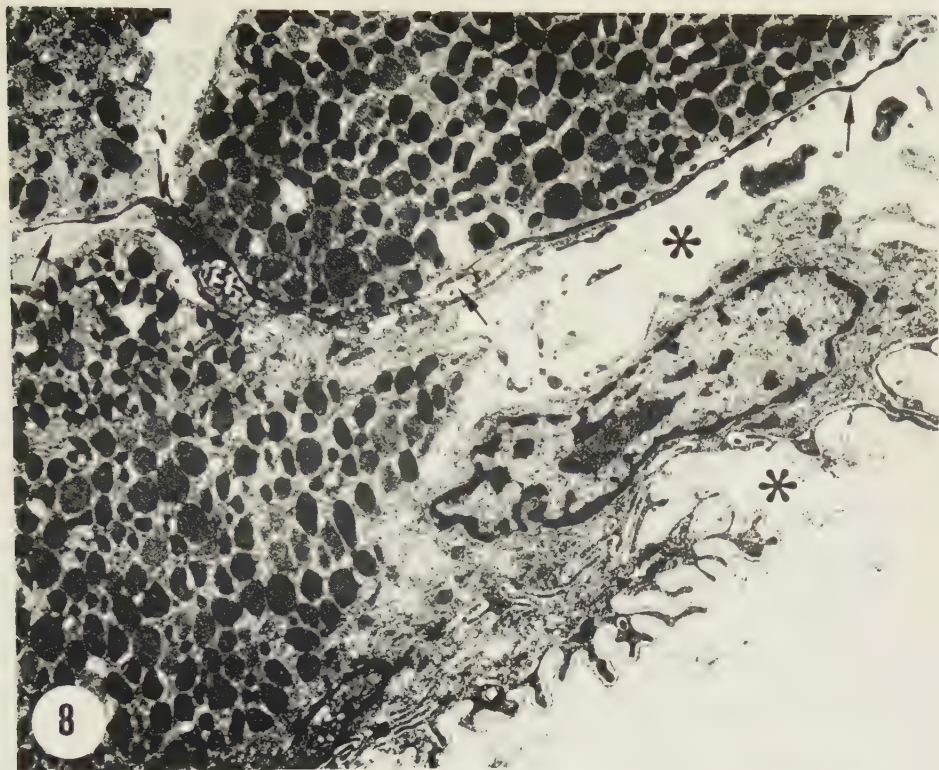
Elimination of neuronal components may proceed for long periods. Ependymal cells (E) contribute to the elimination by engulfing axonal profiles. Host macrophages (M) penetrate into the neural lobe by sending narrow extensions (arrows) into the interstices. They may lie intermingled with extensions from the ependyma (asterisk). They frequently contain residual bodies. x 13 100



FIGURES 8 - 9
=====

Fig. 8. Intermediate lobe (marginal zone) 36 days after implantation. Secretory cells retain their cytologic integrity for long periods, though surrounded by host macrophagic pseudopodia (arrows). Secretory cells sometimes round up and lose their contact with adjacent cells. Formation of collagen fibres is markedly accelerated locally (asterisks).
x 10 600

Fig. 9. Host macrophage exhibiting extensive pseudopodia and numerous residual vacuoles (arrows).
x 9 000



drugs - resulted in a marked increase of the diameter of the skin patch overlying the implant and sometimes even engendered a general skin darkening. A provoked MSH secretion of this kind did not cause a significant, overall loss of secretory granules (Figs. 10, 11).

DISCUSSION

Transection of the hypophysial stalk in Anolis was found by Larsson and Meurling (1979) to increase the MSH release during the first few days. Thereafter, the MSH release was insignificant, although a large number of secretory granules were to be found in the intermediate lobe using electron microscopy. These findings suggest the participation of both inhibitory and stimulatory elements in MSH release regulation. Because it could not be excluded that the hypothalamus and/or the median eminence still exerted some influence on secretory cells of the intermediate lobe, it was desirable to find a site for implantation of neuro-intermediate lobes more distant from direct brain control.

Naturally, the best survival of the implant could be expected by using homografted tissue that was implanted into a well-vascularized area of the body. The relation of the intermediate lobe to the internal carotid arteries obstructs the extirpation of the neuro-intermediate lobe in one piece while keeping the donor alive. Thus, for practical reasons allografted tissue had to be used.

FIGURE 10

=====

Fig. 10. Intermediate lobe 58 days after implantation.

MSH release was stimulated by subcutaneous administration of serotonin 43 min prior to fixation. Secretory cells appear normal for extended periods. They contain vacuoles (asterisk) resembling the cisterns described in intact lobes. PVS, perivascular septum; S, stellate cell; star, atypical vacuole. x 7 600

Inset: Maximal extension of skin patch immediately prior to fixation. Bar indicates 10 mm

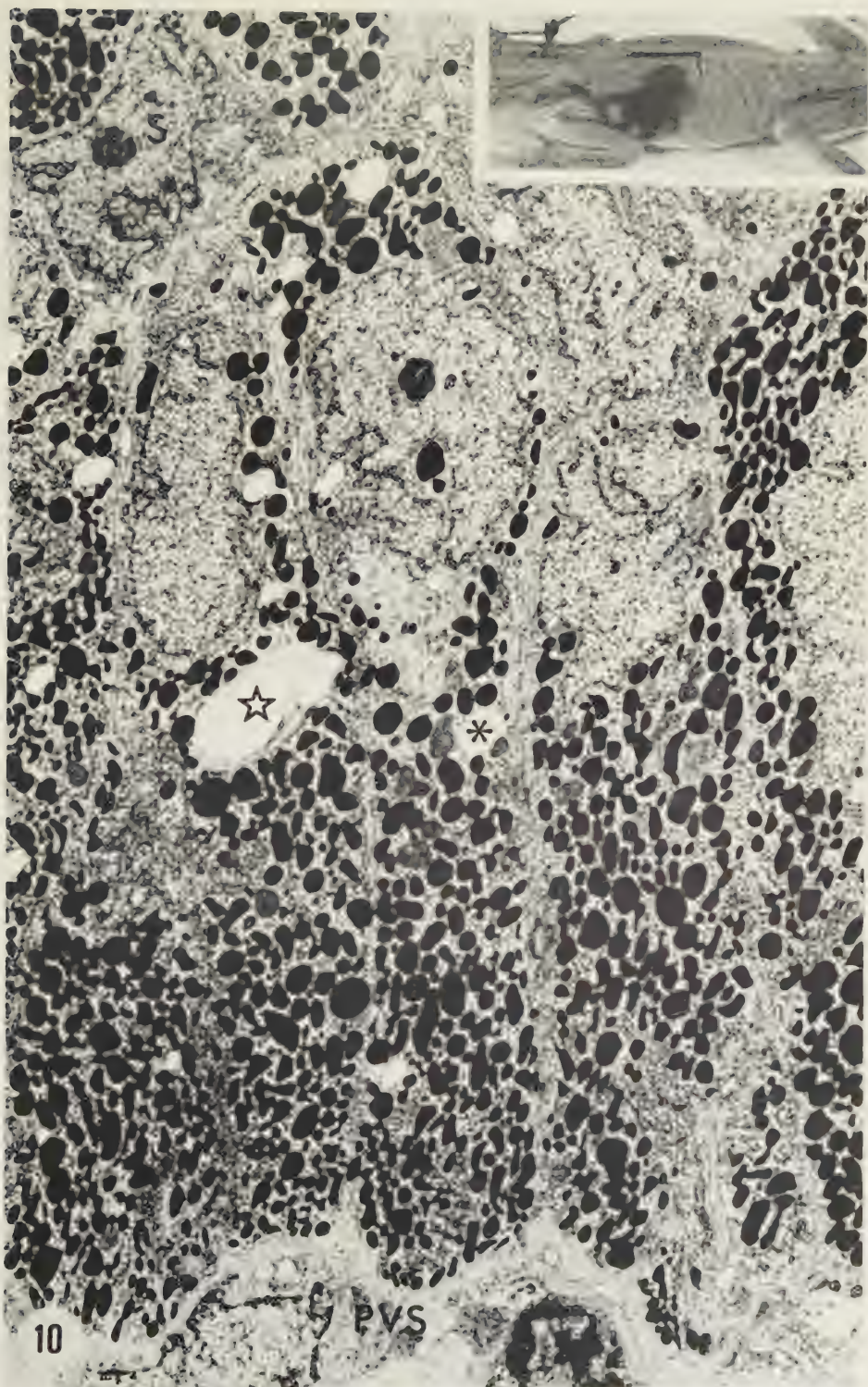


FIGURE 11

=====

Fig. 11. Intermediate lobe, same as figure 10. The MSH release was increased owing to subcutaneous serotonin administration. There was, however, no marked depletion of secretory granules. Secretory cells round up and lose contact with their neighbours (asterisk). PVS, perivascular septum. x 7 800

11

PVS

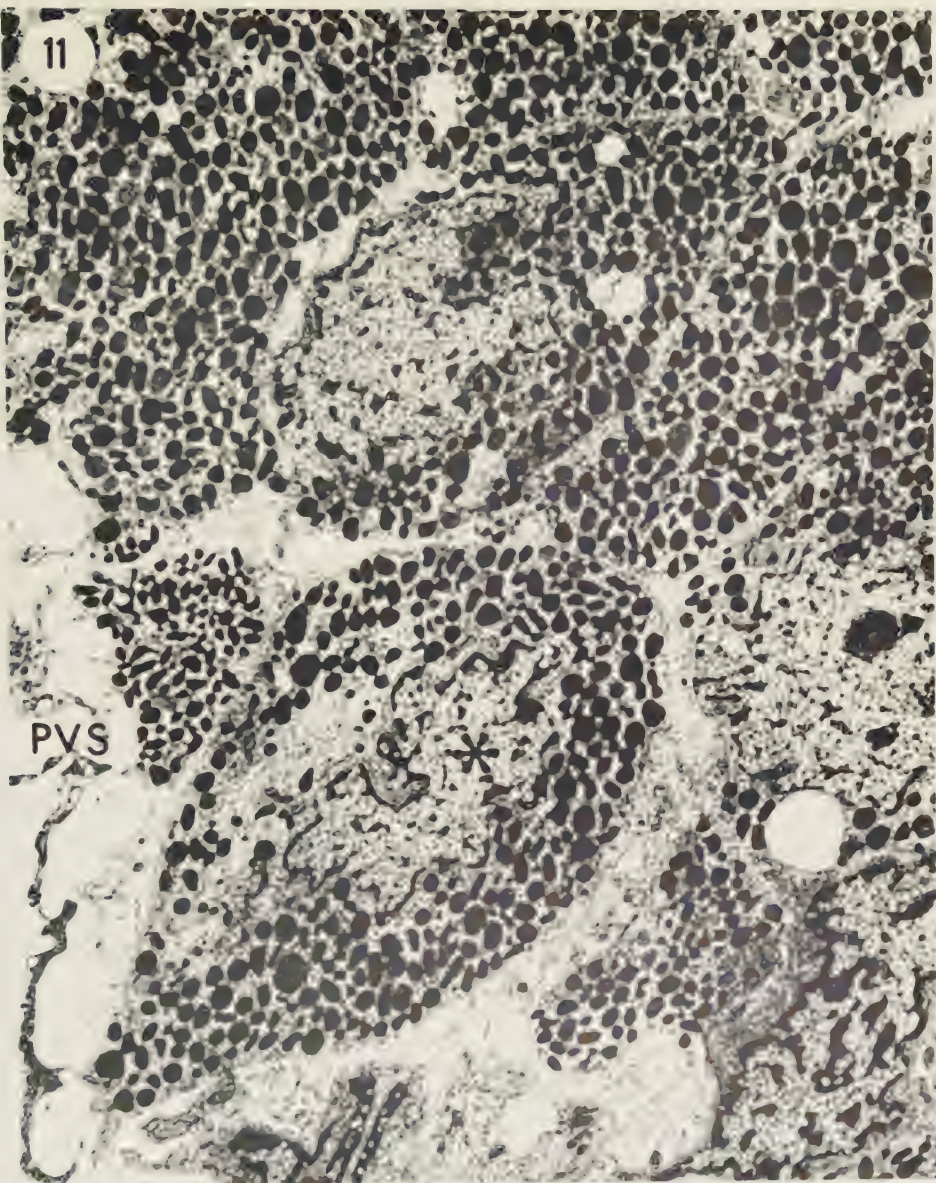


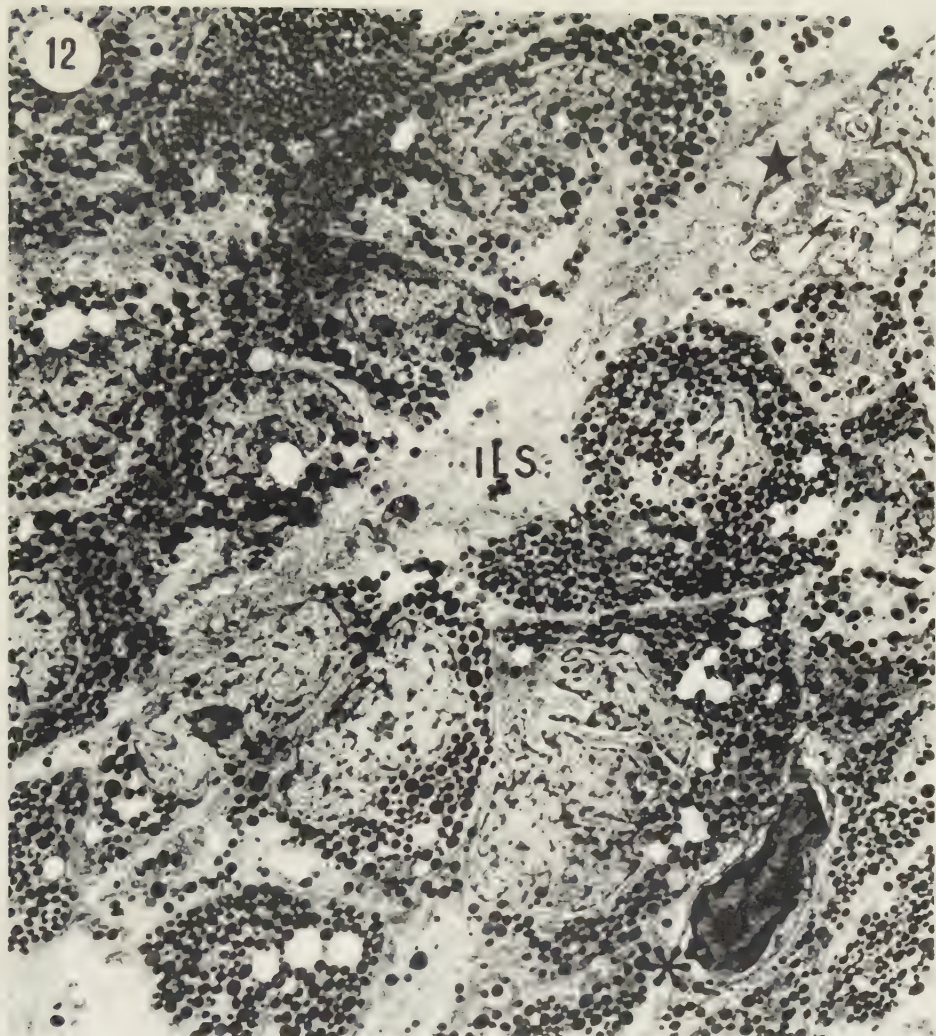
FIGURE 12
=====

Fig. 12. Intermediate lobe 20 days after implantation.

Well preserved secretory cells, heavily loaded with secretory granules surrounding an interlobular septum (ILS). Secretory cell nuclei appear more irregular than normally and the chromatin is denser. The parenchyma occasionally contain host macrophages (asterisk). Stellate cells containing large residual bodies (arrow) indicative of macrophage activity. x 4 400

12

ILS



Considering the finding of Meurling and Larsson (1974) in Anolis that stressful handling caused increased MSH release from implanted neuro-intermediate lobes and the results by Goldman and Hadley (1969) that catecholamines might stimulate the MSH release in Anolis by their β -adrenergic receptors, it was concluded that excessive surgery to the recipient lizard had to be avoided. The choice of a subcutaneous site for the implantation insured minimal distress and very intimate contact between the intermediate lobe and the melanophores of the skin. This close relation between MSH and its effector organ (melanophores) signified that very minute fluctuations in release activity could be discriminated. Implants were also more easily reached by test solutions, reducing the essential dose and thus the risk of severe, systemic reactions. It was assumed that there was a high bioavailability of test solutions to the implant and that pharmacokinetic differences between the test solutions were of nominal importance.

Morphological Considerations

The major disadvantage with the choice of a subcutaneous site for implantation seemed to be the suboptimal blood supply during the first few days. The present study shows that implants were revascularized to a great extent and that there was a survival of major parts of the neuro-intermediate lobe for extended periods. Although a number of secretory cells were eliminated by host macrophages and obviously some areas of the intermediate lobe were atrophied owing to insufficient nutrition, especially during the initial period, the

remaining cell population appeared to be normal. Secretory cells exhibited close cytological similarities with those of in situ conditions. Consequently, there were no morphological signs that the remaining secretory cells were adversely affected until they were reached by macrophages and eliminated. The volume of the intermediate lobe containing intact secretory cells seemed to correlate roughly with the functional capacity.

It could be concluded that immunoreactions or insufficient nutrition only occasionally made experimentation with the intermediate lobe impossible during the period encompassed by the study.

It has been shown that neuro-intermediate lobes could be kept vital under in vitro conditions for less than 1 day (Anolis: Bower and Hadley 1972, Thornton and Geschwind 1975a, Davis and Hadley 1976) or for longer periods (Rana: Semoff et al. 1978). Protein biosynthesis has been shown to continue in the mouse pars intermedia under in vitro conditions (Isherwood and Thornton 1977). Semoff et al. (op. cit.) kept neuro-intermediate lobes in tissue culture for 6 months. They concluded that MSH was continuously secreted for 4 months and that the ultrastructural appearance resembled intact conditions for up to 8 months. Secretory cells of the intermediate lobe exhibit a conspicuous rough endoplasmic reticulum and well-developed Golgi complexes, thus closely resembling cells described by Larsson et al. (1979b) in Anolis during forced MSH release.

Functional Considerations

Most implants exhibited a gradually diminishing capacity for MSH release during the experiment. Some implants, however, responded to a certain stimulus by releasing the same quantity of MSH throughout the experimental period, judging from the size of the resulting skin patch.

The growth of the dark skin patch proceeded in one of two ways upon stimulation based on the degree of revascularization. In a minority of the implants, the skin patch was very small and increased in size only slightly. Instead there was, after stimulation, an immediate drainage of newly released MSH through blood vessels, thus affecting the general skin colour. It could be concluded that spontaneous MSH release was insignificant and that the amount of MSH released upon stimulation was great enough to cause darkening of the entire skin. The implanted neuro-intermediate lobe thus responded in much the same way as the lobe in situ, viz. when moving a lizard with green skin from a light to a dark background (Larsson and Meurling 1979).

Although dark striae radiated from most implants (indicative of blood vessels) after stimulation of MSH release, there were less intimate associations between blood vessels and the implanted tissue in most cases. This caused local accumulation of MSH, which was evident by the growth of the skin patch. It would be reasonable to distinguish two consecutive phases after stimulation of the implant in these cases. The first phase comprised the darkening of the skin patch

and the period of growth until the sharp demarcation between the dark patch and the pale green skin vanished. This first phase is suggested to correspond roughly to the period of rapid MSH release. The largest brown skin patch found averaged 11 mm in diameter.

The successive "blurredness" of the marginal zone of the skin patch obviously indicated a diffusion of MSH to surrounding parts of the skin. Theoretically, a situation would occur when the addition of MSH to the area was balanced by forces that removed MSH - i.e. diffusion, transport by blood vessels, and metabolism - in a steady-state condition. This situation did not ensue, however, for any significant period of time. The implant never maintained a continuous high rate of secretion for longer periods, suggesting the need for continuous stimulation. The second phase was initiated when the demarcation became fuzzy, which was normally followed by a decreased diameter of the dark central part of the skin patch. This suggested a decrease of the MSH concentration in the area and that elimination of MSH overrode the release of new hormone. By studying the dynamics of growth and remission of the skin patch, it was seemingly possible to acquire data on the duration of active MSH release after various treatments.

The amount of MSH needed to darken the skin could be calculated from experiments using pieces of Anolis skin in vitro floating on a Ringer solution with known concentrations of synthetic α -MSH (cf. Björklund et al. 1972). It was found that about .5 ng/ml synthetic α -MSH was needed to induce brown colour in skin pieces from intact animals. Personal observations (unpublished) indicate that the skin's

sensitivity to MSH is slightly increased after a period of hypophysectomy. It is reasonable to suggest that the MSH concentration beneath the supranormal brown skin patch during the initial period of stimulation exceeded .5 ng/ml. The amount of MSH present immediately adjacent to the implant is, however, very likely a function of various factors, such as release rate and elimination by metabolism, diffusion and drainage through blood vessels (cf. dark striae of the skin), of which the latter varied considerably among individual specimens. Consequently, there seemed to be no easy way to calculate the quantity of MSH released by the intermediate lobe by measuring the diameter of the skin patch overlying the implant.

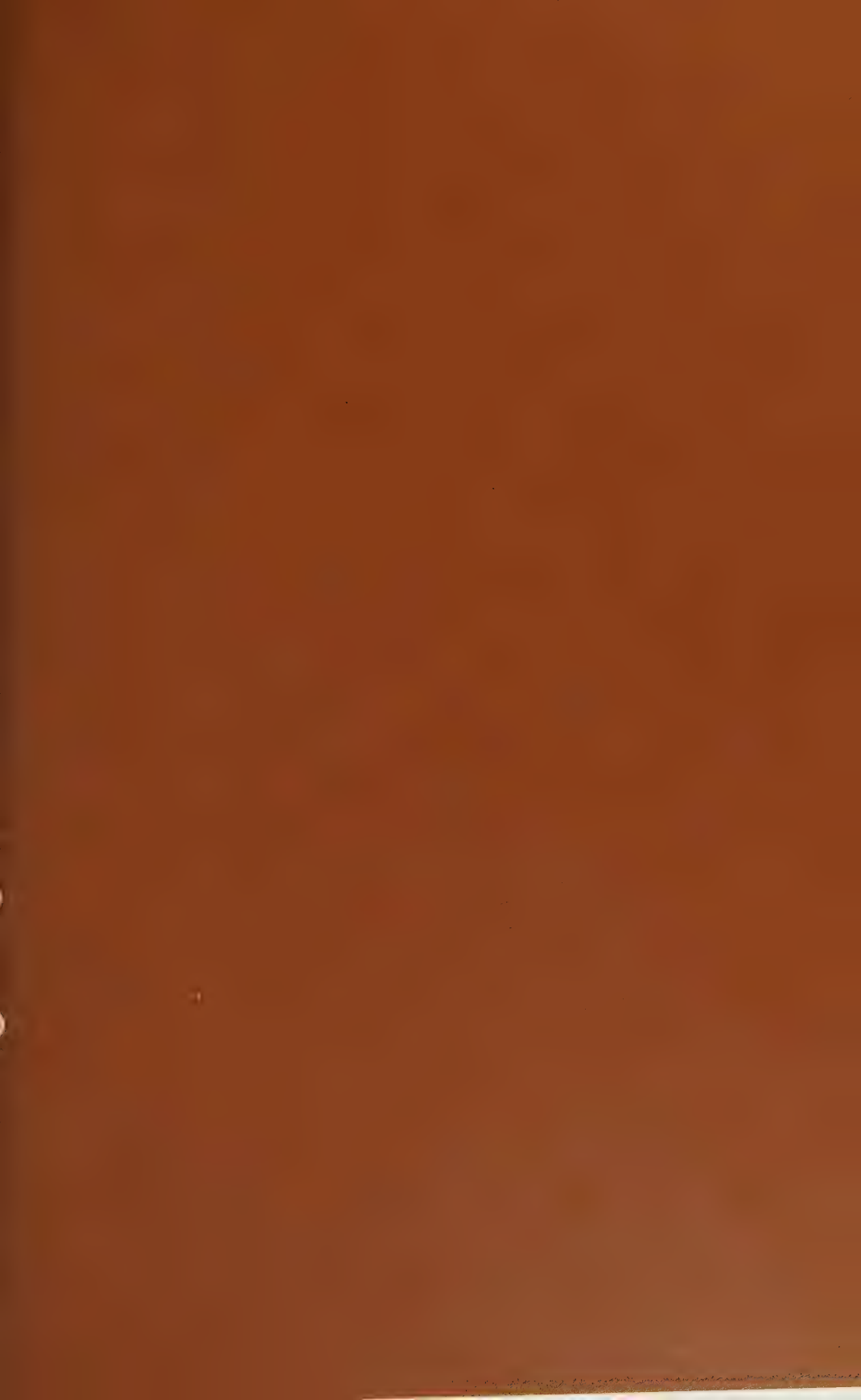
Although the results obtained with the present technique were not directly conveyable into a quantification of released MSH in a way similar to bioassays and RIA methods, it might be a practical method to screen various substances for their potency to affect the rate of MSH release. This study also confirms that allografted secretory cells of the intermediate lobe will survive and secrete MSH during extended periods. The close relation of MSH cells in the implant and melanophores of the skin appeared to be advantageous in spite of suboptimal nutrition of the subcutaneous implant. The technique may therefore appear to be a valuable complement to other methods and to contribute to the understanding of MSH release regulation. The fact that the implants with restored vascular contacts frequently released enough MSH to cause a darkening of the entire skin of the animal suggests that the amount of MSH is on a parity with that found under intact conditions.

REFERENCES

- Björklund A, Meurling P, Nilsson G, Nobin A (1972) Standardization and evaluation of a sensitive and convenient assay for melanocyte-stimulating hormone using Anolis skin in vitro. J Endocrinol 53, 161-169
- Bower A, Hadley Mac E (1972) Ionic requirements for melanophore-stimulating hormone (MSH) release. Gen Comp Endocrinol 19, 147-158
- Davis M D, Hadley Mac E (1976) Spontaneous electrical potentials and pituitary hormone secretion (MSH). 261, 422-423
- Dongen W J van, Jörgensen C B, Larsen L O, Rosenkilde P, Loft B, Oordt P G W J van (1966) Function and cytology of the normal and autotransplanted pars distalis of the hypophysis in the toad Bufo bufo (L). Gen Comp Endocrinol 6, 491-518
- Gibson J D (1979) The origin of the neural macrophage: a quantitative ultrastructural study of cell population changes during Wallerian degeneration. J Anat 129, 1-19
- Goldman J M, Hadley Mac E (1969) In vitro demonstration of adrenergic receptors controlling melanophore responses of the lizard, Anolis carolinensis. J Pharm Exp Therap 166, 1-7
- Hadley Mac E, Hruby V J, Bower Sr A (1975) Cellular mechanisms controlling melanophore stimulating hormone (MSH) release. Gen Comp Endocrinol 26, 24-35
- Isherwood H, Thornton V F (1977) Protein biosynthesis in vitro by the pars intermedia of the mouse pituitary gland. J Endocrinol 72, 173-179

- Iturriza F C (1969) Further evidences for the blocking effect of catecholamines on the secretion of melanocyte-stimulating hormone in toads. *Gen Comp Endocrinol* 12, 417-426
- Karnovsky M J (1965) A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. *J Cell Biol* 27, 137-138A
- Larsson L (1981) Control of the pars intermedia of the lizard, Anolis carolinensis. V. Extravascular transfer and cellular uptake of horseradish peroxidase in the neuro-intermediate lobe. *Cell Tissue Res* 214, 1-22
- Larsson L (Manuscript) Control of the pars intermedia of the lizard, Anolis carolinensis. VII. MSH release from allografted neuro-intermediate lobes under various experimental conditions
- Larsson L, Meurling P (1979) Control of the pars intermedia of the lizard Anolis carolinensis. IV. Transection of the pituitary stalk. Effects on colour change and histology. *Cell Tissue Res* 204, 201-216
- Larsson L, Rodríguez E M, Meurling P (1979a) Control of the pars intermedia of the lizard, Anolis carolinensis. II. Ultrastructure of the intact intermediate lobe. *Cell Tissue Res* 198, 411-426
- Larsson L, Rodríguez E M, Meurling P (1979b) Control of the pars intermedia of the lizard, Anolis carolinensis. III. Changes in ultrastructure of the disconnected neuro-intermediate lobe. *Cell Tissue Res* 199, 1-23
- Levitin H P (1980a) Further evidences that serotonin may be a physiological melanocyte-stimulating hormone releasing factor in lizard Anolis carolinensis. *Gen Comp Endocrinol* 40, 8-14

- Levitin H P (1980b) Monoaminergic control of MSH release in the lizard Anolis carolinensis. Gen Comp Endocrinol 41, 279-286
- Masur S K (1969) Fine structure of the autotransplanted pituitary in the red eft, Notophthalmus viridescens. Gen Comp Endocrinol 12, 12-32
- Meurling P, Larsson L: Pars intermedia control with and without innervation - studies in elasmobranchs and a lizard. In: Neurosecretion - The final neurosecretory pathway. (VI International Symposium on Neurosecretion, London 1973) (F Knowles and L Vollrath eds) pp 198-206. Berlin-Heidelberg-New York: Springer Verlag 1974
- Rodríguez E M, Larsson L, Meurling P (1978) Control of the pars intermedia of the lizard, Anolis carolinensis. I. Ultrastructure of the intact neural lobe. Cell Tissue Res 186, 241-258
- Semoff S, Fuller B B, Hadley Mac E (1978) Secretion of melanophore-stimulating hormone (MSH) in long-term cultures of pituitary neurointermediate lobes. Cell Tissue Res 194, 55-69
- Thornton V F, Geschwind I I (1975a) The characteristics of melanocyte-stimulating hormone release from incubated pituitaries of the lizard Anolis carolinensis. Gen Comp Endocrinol 26, 336-345
- Thornton V F, Geschwind I I (1975b) Evidence that serotonin may be a melanocyte stimulating hormone releasing factor in the lizard, Anolis carolinensis. Gen Comp Endocrinol 26, 346-353



Control of the Pars intermedia of the Lizard,
Anolis carolinensis

VII. MSH release from Allografted Neuro-
intermediate Lobes under Various
Experimental Conditions

Leonard Larsson^{*}

Department of Zoology, University of Lund,
Lund, Sweden

Acknowledgements: The author is indebted to Dr Rolf Håkansson for advice and valuable criticism and to Mr Per Melin at Ferring for generous supply of oxytocin derivatives and to Miss Ylwa Andersson for excellent typing of the manuscript.

^{*}Supported by grants from the Swedish Natural Science Research Council (to Dr P Meurling) and the Royal Physiographic Society of Lund.

Summary. MSH release was studied from neuro-intermediate lobes allografted under the dorsal skin in hypophysectomized lizards, Anolis carolinensis. There was a spontaneous release from the implant during the first day or two. Thereafter, the MSH release was slight, judged from the insignificant size and darkness of the skin patch overlying the graft. Stressful handling and injection of adrenaline (0.3-30 mM) stimulated the release of MSH. This effect could be blocked by propranolol but not by phentolamine. Serotonin (4.7 mM) greatly stimulated the MSH release, whereas the oxytocin derivatives used were ineffective. Dopamine did not inhibit the MSH release during the period of initial spontaneous release. Haloperidol stimulated the release of MSH from the melanotrophs in two implants, grafted for more than 50 days. The meaning of this is not clear. Grafts exhibited sustained vitality, and the MSH release could occasionally be stimulated for more than 4 months. The use of allografted neuro-intermediate lobes in Anolis is proposed as a valuable method for the study of the mechanisms regulating the release of MSH.

Key words: Neuro-intermediate lobe - Allograft - Anolis carolinensis - MSH release - Serotonin - Melanophore response

INTRODUCTION

The regulation of MSH release from the melanotrophs of the intermediate lobe has been studied in a variety of vertebrate species under different experimental conditions. In many vertebrates dopamine is suggested to inhibit the MSH release (Bower et al. 1974, Hadley et al. 1975, 1977, Tilders and Smelik 1977, Levitin 1980b).

The possible participation of oxytocin derivatives in the regulation of MSH release has been discussed by several authors. The end-chain of oxytocin, L-Pro-Leu-Gly-NH₂ (Nair et al. 1971, Celis et al. 1971a), tocinoic acid and tocin amide (Hruby et al. 1972) have thus been suggested as inhibiting factors for MSH release as has Pro-His-Phe-Arg-Gly-NH₂ (Nair et al. 1972), which was isolated from bovine hypothalamus. MSH releasing capacity has been demonstrated with the oxytocin fragment H-Cys-Tyr-Ile-Gln-Asn-OH (Celis et al. 1971b) and with opiate agonists (Celis et al. 1980).

Biogenic amines, released from the adrenals by stress, have been shown to stimulate the release of MSH from rat pituitaries in situ (Kastin et al. 1967, 1980) and from implanted neuro-intermediate lobes in Anolis (Meurling and Larsson 1974). This type of stimulation of MSH release is linked to the activation of beta-adrenergic receptors (Hadley et al. 1975).

Thornton and Geschwind (1975) demonstrated that serotonin and its precursor 5-hydroxytryptophan increased the MSH release from

incubated neuro-intermediate lobes during the first 5 hrs of incubation. The capacity of this amine to stimulate the release of MSH from Anolis pituitaries has been confirmed by Taraskevich and Douglas (1979) and Levitin (1980a, b). Serotonin and its precursors have gained attention as MSH release stimulants also in amphibians and teleosts (Olivereau and Chambolle 1979, Olivereau and Olivereau 1979, Olivereau et al. 1980). Dopamine has been shown to inhibit the spontaneous electric activity registered from intermediate lobe cells in frogs (Davis and Hadley 1976). In Anolis, the period of high spontaneous MSH release following transection of the hypophysial stalk lasted for some hours to maximally 2 days. After this period of high release the melanotrophs of the intermediate lobe retained an abundance of secretory granules (Larsson et al. 1979b).

The MSH/melanophore system allows the continuous registration in vivo of circulating MSH by watching the skin colour of the animal. In the present study, various drugs were tested after subcutaneous injection for their possible direct effects on MSH release.

In the present paper, the MSH releasing capacity of various substances was tested on allografted neuro-intermediate lobes (NIL) implanted under the dorsal skin in Anolis lizards. The study is concerned with two major questions, i.e. whether the MSH release can be stimulated during phases of slow spontaneous release and whether the MSH release can be inhibited during phases of rapid release.

MATERIALS AND METHODS

Neuro-intermediate lobes ($N = 16$) were allografted under the dorsal skin in hypophysectomized Anolis lizards (Figs. 1-6). Distal lobes were grafted separately for control (Fig. 4; see also Larsson VI). Various drugs (Table I) with suggested effect on MSH release were injected subcutaneously. Their ability to increase or decrease the rate of MSH release from the implants was estimated by measuring the mean diameter of the dark skin patch overlying the implant. For details on implantation procedure and evaluation of growth and remission of the dark skin patch, see Larsson VI.

Injections and Stressful Handling

During the experimentation periods, lizards were kept under minimal stress in boxes with white linings. The test solutions (0.02 ml) were injected subcutaneously with a very fine needle 10 mm behind the neuro-intermediate lobe implant. Efforts were made to complete the injection within 10-15 sec with a minimal discomfort for the animals. The test solution caused a small wheal close to the neuro-intermediate lobe implant, but was never allowed to lift the skin above it. The same orifice was used for consecutive injections. Experimentation was undertaken at intervals of two or three days, allowing animals and grafts to recover between experiments. Effects of endogenous stress hormones on the MSH release were studied by intense handling of the animal during 0.5-1 min. The level of exhaustion could be briefly judged from the degree of hyperventilation. Stress caused by the injection procedure per se was evaluated by injection of buffered saline. Concentrations and doses of test solutions are given in Table I.

Test Solutions. Adrenaline, dopamine and 5-HT were dissolved in saline (0.8 % NaCl/0.1 M phosphate buffer (pH 7.4), 9:1) and stabilized with 10 μ M ascorbic acid. The solutions were used fresh. Peptides (oxytocin and its derivatives, see Table I) were dissolved in a small amount of acetic acid (pH 3.4) and diluted in the same saline. Peptides were stored frozen in plastic ampoules at a concentration of 100-300 μ g/ml. They were used immediately after dilution.

Evaluations of General Skin Colour and MSH release from the Implants

General skin colour was assessed according to a five-point scale G (green), BG (brown-green), GB (green-brown), B (brown) and SB (supra normal brown, seen under experimental conditions only). For details of procedure see Larsson and Meurling (1979). After the initial period of overall skin darkening immediately following graft implantation there remained a dark skin patch above the implant (cf. Larsson VI). The size of this dark skin patch, expressed as the mean of the maximal and minimal diameters ($\frac{D_{\max} + D_{\min}}{2}$), the colour of the skin patch, and the presence of radiating striae from the implant were registered every 2nd to 5th min during experimentation. Dark striae indicated blood vessels draining newly released MSH from the implant into the main blood circulation. For details on increase of the dark skin patch after stimulation and the meaning of skin patch size in the evaluation of MSH release, see Larsson (VI). The central area of the skin patch exhibiting brown or supra normal skin colour was measured.

Because handling, and the injection per se, may evoke some discomfort to the animals - leading to release of adrenaline from the adrenal glands with the consequent release of MSH and effects on skin colour (Kleinholz 1938a, b, Hadley and Goldman 1969) - it was of vital importance not to upset the animals during the experiments. To further eliminate the possibility that endogenous amines may cause MSH release, beta-adrenergic receptors were blocked with propranolol (cf. Hadley et al. 1975) about 30 min prior to the injection of the test substance.

Because the capacity for MSH release has been shown to vary both with individual grafts and with the interval of implantation in a rather unpredictable way (Larsson VI), it was found practical to finish each day's experimentation by the administration of a standard dose of serotonin (0.02 ml, 4.7 mM). The "maximal" MSH release capacity could then be estimated from the response to this injection.

Table I. Substances used in the study

Biogenic amines:

Serotonin (5-HT) ⁺	4.7-71 mM	24-355 ng/kg BW
Adrenaline ⁺	0.3-30 mM	1.5-150 ng/kg BW
Dopamine ⁺	5.3-53 mM	26-265 ng/kg BW

Receptor blocking agents:

Phentolamine (Regitin [®])	3.6-27 mM	18-135 ng/kg BW
Propranolol (Frekvén [®])	34 mM	170 ng/kg BW
Haloperidol (Haldol [®])	33 mM	165 ng/kg BW
Cyproheptadin (Periactin [®])	0.9 µM	4.5 µg/kg BW

Oxytocin and its derivatives:

Oxytocin ⁺⁺	40-80 µg/kg BW
Mesotocin ⁺⁺	4-160 µg/kg BW
8-Arg-vasotocin ⁺⁺	40 µg/kg BW
Tocinoic acid ⁺⁺	4-80 µg/kg BW
Pro-Leu-Gly-NH ₂ (PLG-NH ₂) ⁺⁺	40-100 µg/kg BW

⁺ Provided by Sigma

⁺⁺ Provided by Ferring

RESULTS

Stimulation of MSH release

There was a significant spontaneous release of MSH during the initial periods after implantation, evoking a very prominent dark skin patch over the neuro-intermediate lobe implant or even an overall skin darkening. The size of the dark skin patch decreased during the first day (exceptionally the first few days) of implantation, however. Afterwards, there remained a skin patch for extended periods. The size of the dark skin patch, probably reflecting the spontaneous release of MSH, varied individually from less than 1 mm to a few mm. Some implants gave rise to a gradually diminishing dark skin patch throughout the experimental period.

Implanted distal lobes caused a less prominent dark skin patch which decreased in size more rapidly and usually vanished within a week.

Stressful Handling and Adrenaline. It was found that handling the animals, i.e. moving them from the terrarium to the white adaptation box, caused an increased size and an intensified darkness of the skin above the NIL graft. The skin above the distal lobe did not change, however (Fig. 7). Conceivably, the darkness of this latter skin patch is at least partly caused by ACTH (which is also known to cause melanine dispersion) and related peptides deriving from the corticotrophs of the distal lobe. Consequently, stressful handling did not directly affect the release of ACTH. Stressful influence on the lizards has also been shown (Kleinholz 1938a, b) to affect the melanophores directly. They thereby firstly reacted by melanine aggregation (lightening of the skin) and secondly, after severe stress or after high concentrations of adrenaline (30 mM) the melanine was dispersed in some groups of melanophores

scattered all over the skin, leading to the so-called mottling reaction (Figs. 3, 7). The first and second responses have been shown to be due to stimulation of alpha-adrenergic and beta-adrenergic receptors of the melanophores respectively (Goldman and Hadley 1969).

The direct effects on skin after handling was sometimes manifested by a decrease in size and darkness of the skin patch above the distal lobe graft (Fig. 4, 42 min). Frequently, the dark skin patch disappeared. The growth of the dark skin patch above the NIL implant after adrenaline was also counteracted by the direct alpha-adrenergic receptor stimulation of the skin after stressful stimulation, but here the MSH effect was dominant. Effects on the skin of handling or of adrenaline were markedly decreased after propranolol, a beta-adrenergic receptor blocking agent (Figs. 3, 6, 8) (cf. Goldman and Hadley 1969).

The receptor blocking effect of propranolol appeared to be most accentuated between 25 and 35 min after the administration. Propranolol also blocks the beta-adrenergic receptors of the skin. As is shown in figure 3 (25 min) there was a melanine dispersion in some melanophores in the marginal zone of the area not reached by propranolol, indicative of a subtotal beta-adrenergic blockage in this area. The greater concentration of adrenaline (30 mM) caused a marked stimulation of beta-adrenergic receptors even in the area reached by propranolol (Fig. 3, 60-340 min) by competition with the antagonist. There was no further stimulation of the MSH release, however. The injection of propranolol in this experiment

FIGURES 1 - 3

=====

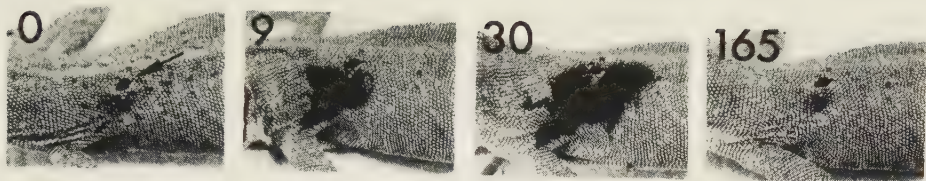
Fig. 1. Lizard no 360, 2 days after implantation. Serotonin (4.7 mM) was injected subcutaneously at time 0, which caused a marked growth of the dark skin patch.

1a: arrow, scar of implantation

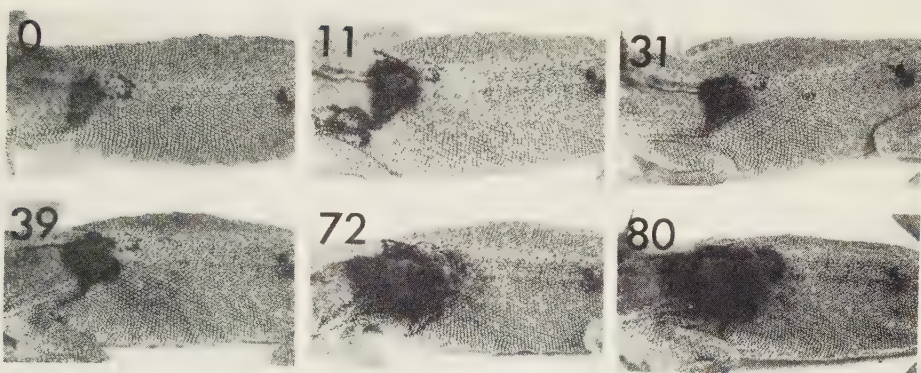
1b: $\bar{D}_m$ mm, mean diameter of the skin patch; overall skin colour was green (O) or brown-green (asterisks)

Fig. 2. Lizard no 401, 3 days after implantation. Phentolamine (27 mM) was administered at 0 min (a); adrenaline (3.0 mM) at 30 min (b), causing a minor stimulation of MSH release from the implant (31-39 min) and serotonin (4.7 mM) at 53 min (c). The latter drug caused a marked increase of the dark skin patch (39-80 min)

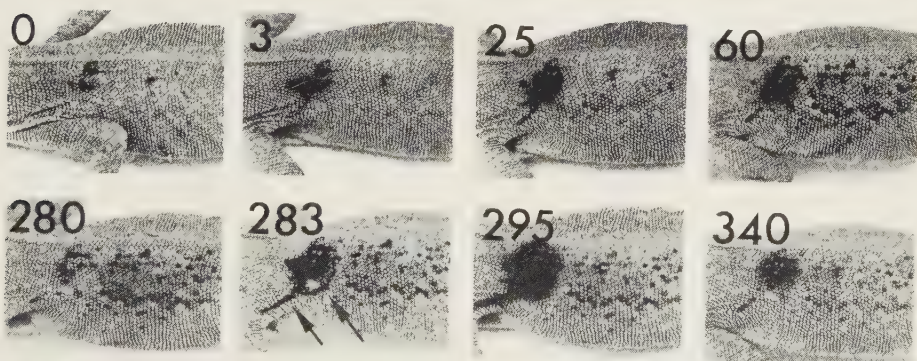
Fig. 3. Lizard no 360, 12 days after implantation. The lizard was given subsequently propranolol (3.4 mM) at 0 min (a) (the lizard was accidentally stressed - partial loss of tail); adrenaline at 20 min (b) and 30 min (c) (0.3 mM and 30 mM, respectively) and serotonin (4.7 mM) at 280 min (d). Adrenaline affected the melanophores of the skin directly (mottling reaction (●) 60-340 min.). There was only a minimal effect of adrenaline on MSH release after propranolol. Darkening of the skin after adrenaline was caused by beta-adrenergic receptors and pallor by alpha-adrenergic receptors respectively. Dark striae (arrows) indicated blood vessels draining newly released MSH from the graft into the main blood circulation.



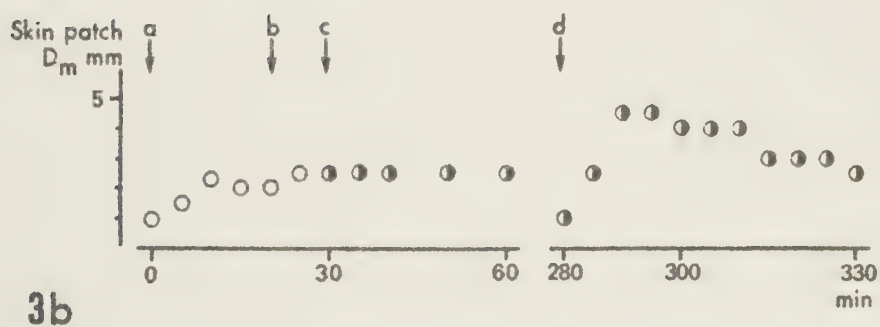
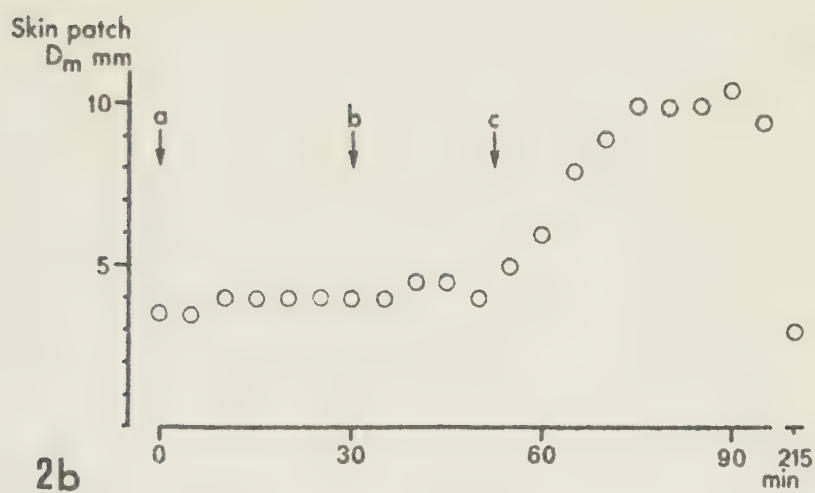
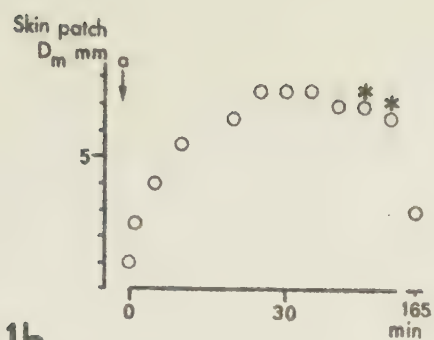
1 : a



2 : a



3 : a



FIGURES 4 - 7

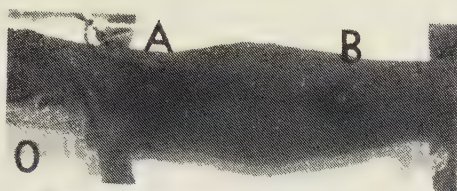
=====

Fig. 4. Lizard no 391 at the day of implantation. There was an excessive MSH release initially (overall brown skin), which could not be inhibited by supplying dopamine (5.3 mM). The handling accomplished with the injection appeared to stimulate MSH release from the implant and to cause a concomitant overall pallor of the skin due to stimulation of alpha-adrenergic receptors of melanophores. A, neuro-intermediate lobe implant; B, distal lobe implant. Fig. 4b. Curve I shows the patch size and the colour of the surrounding skin after dopamine at 0 min (a), and stressful handling at 38 min (b). Skin colours: (●) brown, (▲) green-brown, (□) brown-green and (○) green. Curves II and III likewise illustrate patch sizes and colours of the skin after dopamine in two lizards: 392 at the day of implantation and lizard no 401, 1 day after implantation.

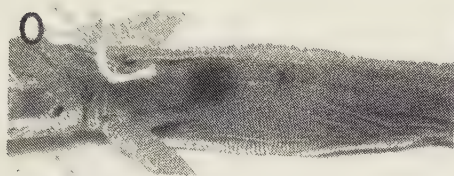
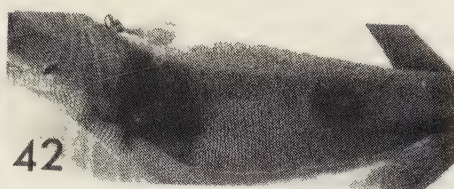
Fig. 5. Lizard no 390, 59 days after implantation. Adding PLG-NH₂ to serotonin (100 µg/kg / 4.3 mM) did not inhibit the release of MSH from the implant.

Fig. 6. Lizard no 402, 13 days after implantation. The lizard received propranolol (3.4 mM) at 0 min (a), haloperidol (33 mM) at 33 min (b), cyproheptadine (0.89 µM) at 66 min (c) and serotonin (4.7 mM) at 96 min (d). Cyproheptadine did not inhibit the effect of serotonin on the implant.

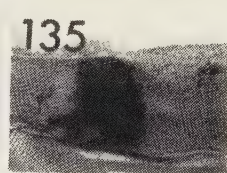
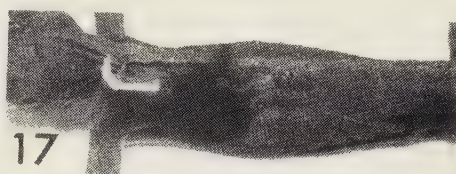
Fig. 7. Lizard no 360, 1 day after implantation. The lizard was exposed to adrenaline (3.0 mM) at 0 min (a) and stressful handling at 180 min (b). MSH release was stimulated to the same extent. Mottling skin appeared after the injection (local effect) (⊗) while the dark skin patch above the distal lobe graft (△) temporarily vanished after handling, due to an overall skin pallor (alpha-adrenergic receptor stimulation of melanophores).



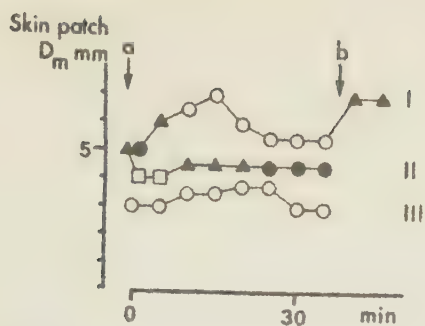
4 : α



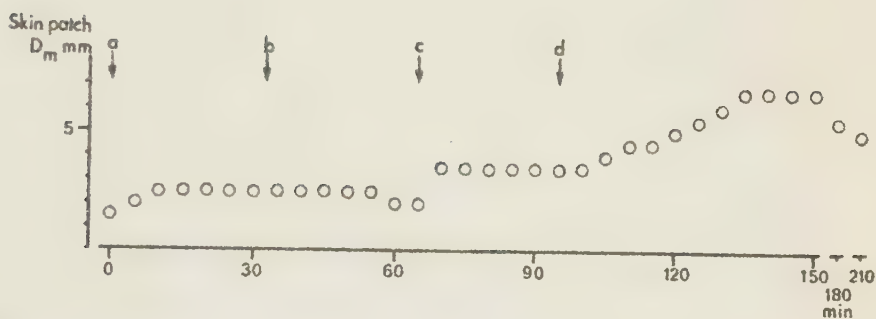
5



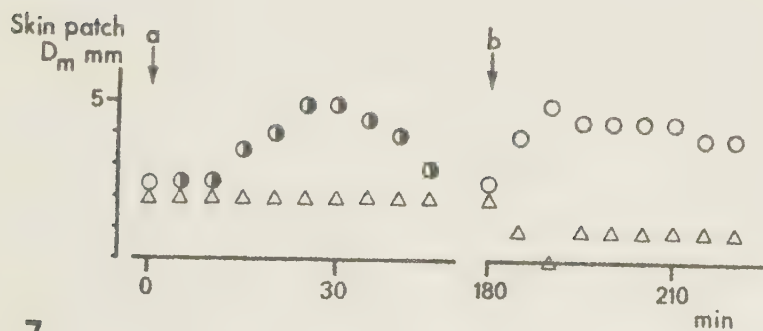
6 : α



4b



6b



7

was probably accompanied by a marked discomfort (partial loss of tail). Stimulation of MSH release therefore occurred at the time of the antagonist administration, before the beta-receptor blockade was effective.

The capacity of phentolamine (3.6-27 mM) (an alpha-adrenergic receptor blocking agent) to inhibit the effect of adrenaline on MSH release was tested in 7 cases. Phentolamine did not inhibit the effects of adrenaline on the melanotrophs.

In one and the same animal adrenaline usually produced the same response as handling (Fig. 7). This response was always less than that caused by 5-HT (Figs. 2, 3, 6, 8 and 9), and there were no signs of exhaustion of secretory cells after the initial period of stimulation, i.e. a second stimulation close to the first one produced almost the same response. Handling or adrenaline seemed to exert less stimulation on MSH release in later periods of implantation (Fig. 8, cf. 5-HT effects Fig. 9).

5-HT. Serotonin was a potent stimulator of MSH release from implanted neuro-intermediate lobes (Figs. 1-3, 5, 6) during the total period of implantation (Fig. 9). The concentrations used produced no direct effects on skin (Table I). In initial periods of implantation, the local accumulation of MSH caused a prominent dark skin patch (Figs. 2, 9). During later periods an overall skin darkening appeared more frequently. This might at least partly be due to a more efficient distribution of MSH to the main blood circulation, by restored bloodvessels to the implant. As 5-HT caused a stimulation of the MSH

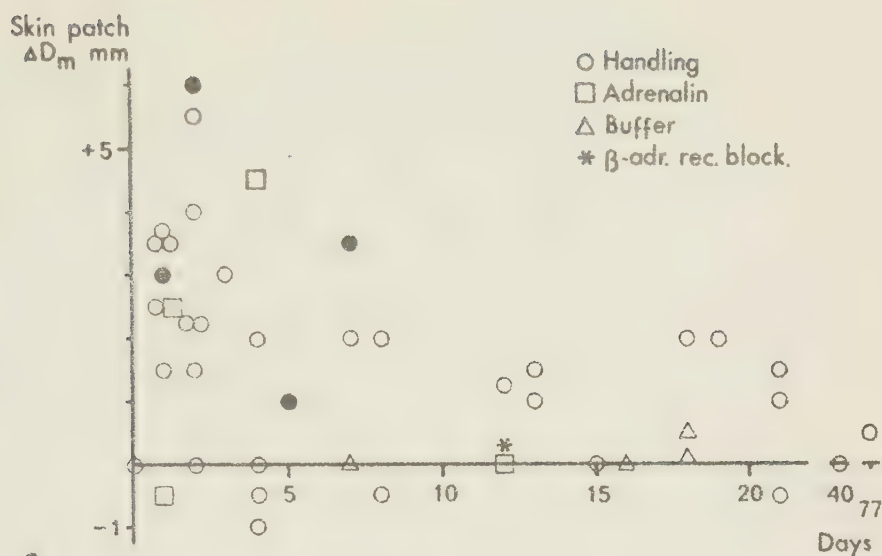
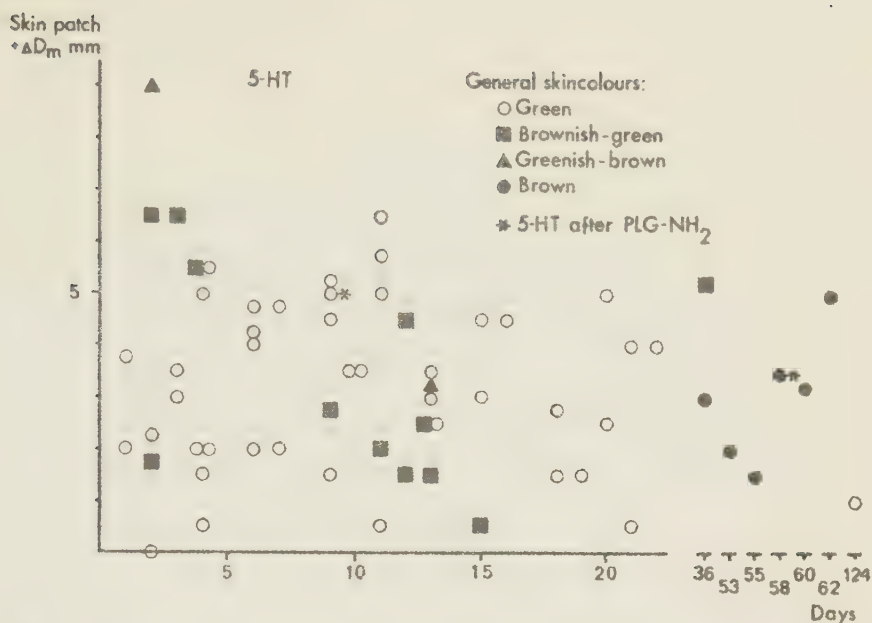


Fig. 8. Changes in skin patch size (ΔD_m mm) after stressful stimulation and adrenaline. Increase of MSH release sometimes caused a darkening of the entire skin (●, green-brown). Alpha-adrenergic receptor stimulation of skin melanophores, caused a decrease of the skin patch size (values below 0)



9

Fig. 9. Increase in size of skin patch ($+\Delta D_m$ mm) after serotonin (5-HT) at various intervals after implantation. A total of 63 registrations are shown. The capacity to stimulate MSH release is as evident in later stages of implantation as during initial stages (cf. Fig. 8)

release, sufficient to cause a generalized skin darkening, the amount of MSH released from implants appeared to be in parity with the amount released in intact specimens on a dark background (cf. Larsson and Meurling 1979).

Growth of the dark skin patch after 5-HT was significantly ($p < 0.001$) slower than after adrenaline or handling. The time delay between injection and maximal extension of the dark skin patch was 26 min and 10 min (means) respectively. The concentration used averaged 4.3-9 mM, but in rare cases higher concentrations (71 mM) were used to non-responders. There was no further MSH release with the higher concentration, however. An absence of response to 5-HT could be taken as a sign of low vitality of the implant.

To evaluate the actual functional capacity of the implant it was appropriate to finish a series of experiments during one day, by the administration of 5-HT. There were no indications that secretory cells of the intermediate lobe under the referred conditions were depleted of secretory granules (cf. Larsson VI) or responded less powerful after a few periods of stimulated release.

Cyproheptadin (63 mM) (a 5-HT receptor blocking agent) failed to inhibit 5-HT stimulated MSH release from 5 implants within 25-30 min after injection.

Oxytocin and Oxytocin derivatives. Although the dark skin patch increased after oxytocin and some oxytocin derivatives (Fig. 10), pretreatment with propranolol abolished this response. Non-responding implants were proven vital by the use of 5-HT afterwards.



Fig. 10. Changes in skin patch size (ΔD_m mm) after oxytocin and its derivatives, could not be discriminated from effects of stressful handling or adrenaline. After propranolol (asterisks) most implants did not respond

Tocinoic acid, designated as an MSH release inhibiting factor (Hruby et al. 1972), did not affect MSH release from the NIL implants in 2 cases and seemed to stimulate the MSH release in 2 cases (Fig. 10). It should be remarked that propranolol was administered to one of these implants already 55 min prior to tocinoic acid, which might imply a subtotal beta-adrenergic receptor blockage (cf. page 9). Consequently, no significant effects on MSH release could be attributed to tocinoic acid in this study.

PLG-NH₂ (40-100 µg/kg BW), a peptide suggested to have MSH release inhibiting effects (Celis et al. 1971a, Nair et al. 1971), was administered concomitantly with 5-HT in two cases (Fig. 9). PLG-NH₂ failed to decrease the response to serotonin.

Haloperidol. Haloperidol (a dopamine receptor blocking agent) was supplied to 7 implants after propranolol, between the 4th and the 58th day after implantation. It was administered during periods of slow spontaneous MSH release. The MSH release was increased in 4 implants, while 3 did not respond. The stimulation of MSH release in 4 implants might be referred to suboptimal beta-adrenergic blockade in one case, another implant was slightly affected while two were markedly affected. All implants responded to subsequent 5-HT, however. Stimulation of MSH release after haloperidol seemed to be most evident in those two implants being grafted for more than 50 days.

Inhibition of Spontaneous MSH release

Dopamine has been suggested to be the MSH release inhibiting factor (MIF) in many vertebrates, including Anolis (Levitin 1980b). In the present study, dopamine was supplied to 4 lizards on the day of implantation or the day afterwards, when the spontaneous MSH release was high. There were no effects on MSH release of dopamine (5.3 mM) in these implants, however (Fig. 4b). In 3 out of 4 animals dopamine affected directly those areas of the skin reached by the amine (Fig. 4b, I and II).

Table II. Summary of effects on MSH release from allografted neuro-intermediate lobes

Handling, stress	marked increase
Adrenaline	marked increase
Serotonin	very marked increase
Oxytocin derivatives	dubious, probably none

DISCUSSION

Several attempts have been made to clarify the mechanisms regulating the release of the melanophore stimulating hormone (MSH) among vertebrates. There is a general opinion among authors that these cells are liable to a tonic, inhibitory control. There is, however, increasing evidence that there are also stimulatory mechanisms involved in MSH regulation, at least in some species. Further, the regulation of hormone release from prolactotrophs and melanotrophs appears to have many things in common. Dopamine seems to exert a release inhibiting effect on both prolactin (PRL) (cf. Frantz 1979) and MSH (Hadley et al. 1975) under in vivo as well as in vitro conditions. Dopamine and some of its agonists thus seem to exert their effect directly on the melanotrophs. Serotonin (5-HT), and its precursor 5-hydroxytryptophan, appear to stimulate release of both hormones, at least in some species (PRL and MSH: (eel) Olivereau and Chambolle 1979, Olivereau and Olivereau 1979, Olivereau et al. 1980; PRL: (rat) Advis et al. 1979, (man) rev. see Frantz 1979; MSH: (Anolis) Thornton and Geschwind 1975, Taraskevich and Douglas 1979, Levitin 1980a, b). There has also been reports that the stimulating action of 5-HT and 5-hydroxytryptophan is mediated by the prolactin releasing factor (rat: Garthwaite and Hagen 1979). Endorphin and met-enkephalin have also been reported to stimulate the release of PRL from the rat pituitary (Dupont et al. 1977, Rivier et al. 1977).

Electrophysiological experiments in vitro with prolactotrophs (fish, Alosa pseudoharengus) and melanotrophs (rat and Anolis) (Taraskevich and Douglas 1979) have demonstrated Ca^{2+} dependent action potentials from secretory cells. Interestingly, the character of action potentials differed between the three species, viz, they are more frequent in fish prolactotrophs and rat melanotrophs than in Anolis melanotrophs. It was further shown that 5-HT markedly stimulated action potentials in Anolis, caused a diminished frequency of action potentials in the fish, and left the action potential pattern in rats almost unaffected. It should be mentioned here that Olivereau and Olivereau (1979) in contrast found a marked activation of PRL cells in the eel after 5-HT.

Taraskevich and Douglas (op. cit.) further stated that Na^+ appears to play a major role in the action potentials of rats, whereas Ca^{2+} and not Na^+ seem to be essential in Anolis. Na^+ , however, appears to have a major role in the response to 5-HT of secretory cells in Anolis. Ca^{2+} dependent action potentials have been shown also in the frog Rana pipiens (Davis and Hadley 1976).

The possibility that different mechanisms regulate the release of MSH in different species makes it inadvisable to draw universal conclusions from studies on one species only. Some of the confusion concerning the importance of different hypophysiotrophic factors may derive from such misunderstandings. In fact there may be different responses to a proposed melanotrophic factor even in

closely related species. Thus, the ring structure of oxytocin and its amide (tocinoic acid and tocin amide) has been alleged to exhibit interspecific differences in their effect on MSH release, displaying MSH release inhibiting factor (MIF) activity in the rat and hamster, in Rana catesbiana and Bufo marinus, but strikingly not in Rana pipiens (Hruby et al. 1972, Hadley et al. 1973). Grant et al. (1973) failed, however, to demonstrate any such effect on MSH release of either tocinoic acid or the side-chain of oxytocin Pro-Leu-Gly-NH₂ (PLG-NH₂) (Thody et al. 1980) in the rat in vitro.

There has been an apparent dispute in the literature about the importance of small peptides on the MSH release regulation. Nair et al. (1971) and Celis et al. (1971a) thus claimed that PLG-NH₂, isolated from bovine hypothalamus, constitutes an MSH release inhibiting factor (MIF), while Hadley et al. (1973) were unable to demonstrate an MSH release inhibiting effect of this peptide in the frog and in mammals. Neither could Thornton and Geschwind (1975) find any such effect in Anolis. The 1-5 peptide of oxytocin (H-Cys-Tyr-Ile-Gln-Asn-OH) has been postulated to be stimulatory to MSH release (MSH-RF) (Celis et al. 1971b). Another peptide Pro-His-Phe-Arg-Gly-NH₂, isolated from bovine hypothalamus was suggested by Nair et al. (1972) to constitute a second MIF parallel to PLG in the frog. These findings are, however, conflicting and some authors have been rather critical to the involvement of peptides in MSH release regulation (Tilders and Smelik 1977, Hadley and Hruby 1977).

Although the number of tests in the present investigation with oxytocin and its derivatives was too small to allow conclusive

statements, there was no evidence that these peptides affect the MSH release in a more significant manner in Anolis.

An important factor to consider in the study of MSH release regulation in vivo is the importance of circulating amines released as a consequence of stressful handling (cf. Levitin 1980b). Although the effects of adrenaline on the MSH release are not as obvious as the response to 5-HT, the response is great enough to produce misleading results when testing different factors with proposed significance in MSH release regulation. The present study illustrates the necessity to perform the experiments with a minimum of discomfort to the animals. However, it appeared essential also to block the beta-adrenergic receptors in order to further assure that the results are not biased by stressing the animals.

A stressing influence on intact Anolis never leads to an overall darkening of the skin. This is obviously due to the fact that adrenal amines exert their action also directly on the skin (Kleinholz 1938a, b, Hadley and Goldman 1969), causing a skin pallor in the presence of normal levels of circulating MSH. The direct effects on the skin was in this case more dominant than the effects on the melanotrophs. These conflicting effects of adrenaline injections could be demonstrated with the present method. Injection of adrenaline close to the graft affects primarily the graft (giving rise to a marked increase of the MSH release and a growing dark skin patch overlying the implant).

Stressful handling (which affects the entire animal) stimulated the MSH release as evidenced by a growing dark skin patch over the NIL implant, but concomitantly there was an overall pallor of the skin, which was manifested by the disappearance of the dark skin patch over the DL implant (which was not stimulated by the handling) (Fig. 7).

The very local effect of injected solutions is further illustrated by the finding that propranolol injections blocked the beta-adrenergic receptors of the skin very locally. Skin effects after subsequent adrenaline injections varied with the distance from the area reached by propranolol (Fig. 3a). In central parts of this area, adrenaline caused a pallor of the skin, while in more peripheral parts beta-adrenergic receptors were stimulated, leading to the mottling reaction. Further distally, adrenaline had no effect at all. Only after very high concentrations of DL-dichloroisoproterenol (beta-adrenergic agonist, 40 mM) (unpublished) the effect on the skin was generalized and also causing a darkening of the so-called postorbital spot (Kleinholz 1938a). The fact that the effects of injected test solutions might indeed cause systemic reactions was evident after the administration of high concentrations of carbachol (0.1 μ M). This acetylcholin agonist caused defecation (unpublished).

The conflicting result obtained here that dopamine (DA, considered to inhibit MSH release in various vertebrates cf. Hadley et al. 1977) and a dopamine agonist, apomorphin (unpublished), administered to the graft during the initial period of great spontaneous MSH release, failed to inhibit the MSH release, is supported by the findings of Levitin (1980b)

that DA did not inhibit the spontaneous basal MSH release, although DA exerted a marked inhibition on the 5-HT induced stimulation.

The antipsychotic drug pimozide (DA receptor blocking agent) caused a darkening of the skin in intact Anolis (Levitin 1980b), which suggests an influence of DA on MSH release in vivo. Interestingly, para-chlorophenylalanine (an inhibitor of tryptophan hydroxylase - leading to depletion of 5-HT) completely abolished the response to pimozide. This indicates that both DA and 5-HT are important in MSH release regulation in vivo.

Various antipsychotics (with DA receptor blocking effect) cause MSH release in vivo (Kastin and Schally 1966, Scott and Stillings 1972), and galactorrhea (increased PRL secretion) is one of the known adverse effects with antipsychotic drugs (see Frantz for rev. 1979). These effects seem to be confined to in vivo conditions with the hypophysis in situ and connected to the brain.

In the present study, haloperidol (an antipsychotic drug) had no clear influence on MSH release. It is suggested that haloperidol exerts its action on MSH release through effects on DA fibres in vivo. It could not be totally excluded, however, that the insignificant MSH release during later stages of implantation was due to an influence of circulating amines on the MSH release of the implant (through an alpha-adrenergic receptor stimulation cf. Hadley et al. 1975). One can only speculate about the significance of the varying responses to haloperidol in this study. Although the scarce number

of experiments here do not allow conclusive statements, it is worth mentioning that two of the implants that were stimulated by haloperidol were grafted for more than 50 days. The meaning of this is not clear, but theoretically the implant might be preferentially exposed to circulating amines during later stages of implantation due to improved blood circulation. There is also the possibility that aminergic receptors on the melanotrophs might have become super-sensitive as a consequence of sustained deprivation of impulses, leading to an increased response to circulating amines. Haloperidol might act as an alpha-adrenergic receptor blocker and block the response to circulating amines of the melanotrophs. These hypotheses do not, however, provide a satisfactory explanation on the rapid cessation of spontaneous MSH release after transplantation.

The failure of cyproheptadine ($0.9 \mu\text{M}$) to inhibit the response of subsequent administration of 5-HT was puzzling, because the same drug was shown by Levitin (1980a) to be an effective 5-HT inhibitor in vivo. Perhaps, the rather short time of exposure to cyproheptadine used here (30 min) was insufficient to entirely block the 5-HT receptors (cf. Levitin 1980a, more than 3 h). There is also the possibility that the 5-HT concentration was too great to admit cyproheptadine to block 5-HT receptors effectively. 5-HT injections proved to be a very reliable test on the capacity of MSH release from the grafted neuro-intermediate lobes. The different character of MSH release stimulation between adrenaline and 5-HT was illustrated by the fact that adrenaline never gave rise to the same prominent skin patch size as 5-HT, whatever the concentration of adrenaline.

The use of allografted neuro-intermediate lobes in a manner as discussed in this paper appears to offer the following advantages: MSH release can be studied in the absence of direct brain influence; very small quantities of MSH can be detected; the dynamics in MSH release can be followed continuously; test solutions can be administered in small amounts, minimizing the systemic effects with the use of a suitable concentration of the test solution; direct effects of test solutions on the skin are readily identified; and various test solutions can be screened for possible effects on MSH release in a rather non-laborious way.

The findings support the opinion that MSH release in Anolis is under the influence of stimulating factors, of which 5-HT appears to be a potent one. Further, spontaneous release of MSH seems to be insignificant or at least of limited duration after deafferentation of the neuro-intermediate lobe. Data is accumulating that MSH release regulation is a complex matter, the mechanisms of which may vary between different species. Further, MSH is found in various parts of the brain and the cerebrospinal fluid (Thody et al. 1979). The source of this MSH does not appear to be the hypophysis. Probably MSH has also specific actions within the central nervous system.

REFERENCES

- Advis J P, Simpkins J W, Bennett J, Meites J (1979) Serotonergic control of prolactin release in male rats. *Life Sci* 24, 359-366
- Bower A, Hadley Mac E, Hruby V J (1974) Biogenic amines and control of melanophore stimulating hormone release. *Science* 184, 70-72
- Celis M E (1980) Effects of different opiate agonists on melanocyte-stimulating hormone release: in vivo and in vitro studies. *Can J Physiol Pharmacol* 58, 326-329
- Celis M E, Taleisnik S, Walter R (1971a) Regulation of formation and proposed structure of the factor inhibiting the release of melanocyte-stimulating hormone. *Proc Natl Acad Sci USA* 68, 1428-1433
- Celis M E, Taleisnik S, Walter R (1971b) Release of pituitary melanocyte-stimulating hormone by the oxytocin fragment H-cys-tyr-ile-gln-asn-OH. *Biochem Biophys Res Commun Chem Pathol Pharmacol* 45, 564-569
- Davis M D, Hadley Mac E (1976) Spontaneous electrical potentials and pituitary hormone (MSH) secretion. *Nature* 261, 422-423
- Dupont A, Cusan L, Labrie F, Coy D H, Li C H (1977) Stimulation of prolactin release in the rat by intraventricular injection of beta-endorphin and methionine-enkephalin. *Biochem Biophys Res Commun* 75, 76-82
- Frantz A G (1979) Prolactin. In: *Endocrinology* (L J DeGroot ed) Grune & Stratton, Inc
- Garthwaite T L, Hagen T C (1979) Evidence that serotonin stimulates a prolactin-releasing factor in the rat. *Neuroendocrinol* 29, 215-220

- Goldman J M, Hadley Mac E (1969) In vitro demonstration of adrenergic receptors controlling melanophore responses of the lizard, Anolis carolinensis. J Pharm Exp Therap 166, 1-7
- Grant N H, Clark D E, Resanoff E I (1973) Evidence that Pro-Leu-Gly-NH₂, tocinoic acid, and Des-Cys-tocinoic acid do not affect secretion of melanocyte stimulating hormone. Biochem Biophys Res Commun 51, 100-106
- Hadley Mac E, Bower Sr A, Hruby V J (1973) Regulation of melanophore stimulating hormone (MSH) release. Yale J Biol Med 46, 602-616
- Hadley Mac E, Davis M D, Morgan C M (1977) Cellular control of melanocyte stimulating hormone secretion. Front Hormone Res 4, 99-104
- Hadley Mac E, Goldman J M (1969) Physiological color changes in reptiles. Am Zoologist 9, 489-504
- Hadley Mac E, Hruby V J (1977) Neurohypophysial peptides and the regulation of melanophore stimulating hormone (MSH) secretion. Am Zoologist 17, 809-821
- Hadley Mac E, Hruby V J, Bower Sr A (1975) Cellular mechanisms controlling melanophore stimulating hormone (MSH) release. Gen Comp Endocrinol 26, 24-35
- Hruby V J, Smith C W, Bower S A, Hadley Mac E (1972) Melanophore stimulating hormone: Release inhibition by ring structures of neurohypophysial hormones. Science 176, 1331-1332
- Kastin A J, Arimura A, Viosca S, Barrett L, Schally A V (1967) MSH activity in pituitaries of rats exposed to stress. Neuroendocrinol 2, 200-208
- Kastin A J, Schally A V (1966) MSH activity in pituitary glands of rats treated with tranquilizing drugs. Endocrinol 79, 1018-1020

- Kastin A J, Schally A V, Kostrzewa R M (1980) Possible aminergic mediation of MSH release and of the CNS effects of MSH and MIF-I. *Fed Proc* 39, 2931-2936
- Kleinholz L H (1938a) Studies in reptilian colour changes II. The pituitary and adrenal glands in the regulation of the melanophores of Anolis carolinensis. *J Exp Biol* 15, 474-491
- Kleinholz L H (1938b) Studies in reptilian colour changes III. Control of the light phase and behaviour of isolated skin. *J Exp Biol* 15, 492-499
- Larsson L (Manuscript) Control of the pars intermedia of the lizard, Anolis carolinensis. VI. MSH release from allografted neuro-intermediate lobes and fine structure of the implanted tissue
- Larsson L, Meurling P (1979) Control of the pars intermedia of the lizard Anolis carolinensis. IV. Transection of the pituitary stalk. Effects on colour change and histology. *Cell Tissue Res* 204, 201-216
- Larsson L, Rodríguez E M, Meurling P (1979) Control of the pars intermedia of the lizard, Anolis carolinensis. III. Changes in ultrastructure of the disconnected neuro-intermediate lobe. *Cell Tissue Res* 199, 1-23
- Levitin H P (1980a) Further evidence that serotonin may be a physiological melanocyte-stimulating hormone-releasing factor in the lizard, Anolis carolinensis. *Gen Comp Endocrinol* 40, 8-14
- Levitin H P (1980b) Monoaminergic control of MSH release in the lizard Anolis carolinensis. *Gen Comp Endocrinol* 41, 279-286

- Meurling P, Larsson L: Pars intermedia control with and without innervation - studies in elasmobranchs and a lizard. In: Neurosecretion - The final neurosecretory pathway. (With International Symposium on Neurosecretion, London 1973) (F Knowles and L Vollrath eds) pp 198-206. Berlin-Heidelberg-New York: Springer Verlag 1974
- Nair R M G, Kastin A J, Schally A V (1971) Isolation and structure of hypothalamic MSH release-inhibiting hormone. *Biochem Biophys Res Commun* 43, 1376-1381
- Nair R M G, Kastin A J, Schally A V (1972) Isolation and structure of another hypothalamic peptide possessing MSH-release-inhibiting activity. *Biochem Biophys Res Commun* 47, 1420-1425
- Olivereau M, Chambolle P (1979) Serotonergic control of MSH secretion in the eel. Ultrastructural study after 5-hydroxytryptophan treatment. *J Physiol Paris* 75, 109-112
- Olivereau M, Olivereau J (1979) Effect of serotonin on prolactin- and MSH-secreting cells in the eel. Comparison with the effect of 5-hydroxytryptophan. *Cell Tissue Res* 196, 397-408
- Olivereau M, Olivereau J M, Aïmar C (1980) Responses of MSH and prolactin cells to 5-hydroxytryptophan (5-HTP) in amphibians and teleosts. *Cell Tissue Res* 207, 377-385
- Rivier C, Vale W, Ling N, Brown M, Guillemin R (1977) Stimulation in vivo of the secretion of prolactin and growth hormone by beta-endorphin. *Endocrinol* 100, 238-241
- Scott G T, Stillings W A (1972) Evidence for the release of MSH by phenothiazine ataraxics. *Endocrinol* 90, 545-551

- Taraskevich P S, Douglas W W (1979) Stimulant effect of 5-hydroxy-tryptamine on action potential activity in pars intermedia cells of the lizard Anolis carolinensis: contrasting effects in pars intermedia of rat and rostral pars distalis of fish (Alosa pseudoharengus). Brain Res 178, 584-588
- Thody A J, Lever de Vries C H, Tilders F J (1980) The failure of L-propyl-L-leucylglycinamide to inhibit the release of alpha-melanocyte-stimulating hormone in the rat. Acta Endocrinol (Copenh) 93, 300-305
- Thody A J, Rotte A A de, Wimersma Greidanus T J van (1979) Plasma and cerebrospinal fluid alpha-MSH levels in the rat after hypophysectomy and stimulation of pituitary alpha-MSH secretion. Brain Res Bull 4, 213-216
- Thornton V F, Geschwind I I (1975) Evidence that serotonin may be a melanocyte stimulating hormone releasing factor in the lizard, Anolis carolinensis. Gen Comp Endocrinol 26, 346-353
- Tilders F J H, Smelik P G (1977) Direct neural control of MSH secretion in mammals: The involvement of dopaminergic tuberohypophysial neurones. Front Hormone Res 4, 80-93



Hoc erat in votis

Credite posteri

